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INHERITANCE OF SEED CHARACTERS IN WATERMELON [1]

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INTRODUCTION

Although seed characters have nothing to do with the flavor and texture of flesh of the watermelon (*Citrullus vulgaris* Schrad.) they are of considerable importance in determining its appearance. The belief is widespread that black seeds denote complete maturity and high sugar content; yet many important commercial varieties have white, brown, tan, or speckled seeds. Despite the availability of several different seed sizes, there seems to be a definite preference for large seeds.

Relatively little information is available concerning inheritance in the watermelon; however, intensive breeding operations are under way in many States, and the need for genetic data has become increasingly urgent. Genetic information, in addition to its botanical value, is essential for the most rapid breeding progress. The investigations herein reported are confined to studies on the inheritance of seed length and seed-coat color.

REVIEW OF LITERATURE

No published reference to genetical analysis of seed-length factors has been found. Weetman (*9*) [2] approximated such studies in populations derived from a cross between races wherein weight samples of 25 seeds were light (1.22 ± 0.01 gm.) and heavy (2.60 ± 0.05 gm.). In the F_2 and backcrosses to both parents the lighter weight parent approached monogenic dominance over the heavier, but chi-square analysis failed to establish an acceptable fit between his observed and calculated ratios.

Kanda (*3*) crossed flat black with stippled black and demonstrated as far as F_3 that flat is dominant to stippled by a single-factor difference. A second cross, which also included flat vs. stippled as well as several other characters and for which he postulated four pairs of genes, was carried to an F_2 population of 123 plants. A chi-square analysis of his data shows a significant deviation from this hypothesis, as will be discussed in an appropriate section. McKay (*4*) investigated two crosses, tan × red and green × red, the former phenotype in each cross being dominant to the latter by a single-gene difference. Porter (*8*) demonstrated a single-gene difference of black dominant over tan and tan dominant over white, with the backcross of black × tan to tan giving

[1] Received for publication February 28, 1941. These studies were initiated in 1930 by D. R. Porter at Davis, Calif. Certain F_1, F_2, and backcross populations were grown there until 1935. Beginning in 1936, the investigations were conducted by the U. S. Department of Agriculture at the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.

[2] Italic numbers in parentheses refer to Literature Cited, p. 456.

1 black to 1 tan. Weetman (*9*) investigated three crosses involving the phenotypes which in our study are called black × clump and tan × clump. In the first case black was dominant to tan by one gene, but in the second case the F_1 phenotype was black and the F_2 segregated into 9 black to 3 tan to 3 clump to 1 white-tan-tip (according to the nomenclature adopted in the present study). In the last case Weetman demonstrated that F_1 × tan gives 1 black to 1 tan, and F_1 × clump gives 1 black to 1 clump.

MATERIALS AND METHODS

Inbred parent stocks used for the crosses were rendered as homozygous as possible before hybridization, but since some inbred lines were selected for specific characters (sugar content, etc.) occasional F_2 populations analyzed for seed characters disclosed a few lines (Sun Moon and Stars and Peerless) that were still heterozygous for seed-color genes. Among the different stocks and varieties of *Citrullus vulgaris*, seed length ranges from 5.5 to 15.5 mm. and seed-coat colors range from an almost pure white through red, green, tan, mahogany, and black, with various patterns superimposed. Seed length and seed-coat colors for varieties used in this study are indicated in table 1. The Sun Moon and Stars variety is represented by one strain in which seed pigment is clumped and another in which it is white-tan-tip-colored; Peerless has one strain with white-tan-tip seeds and another with white-pink-tip seeds. The Striped Klondike variety also showed unstable colors, but the strain used as parent in the studies herein reported showed only clump, a phenotype varying in segregating populations from a large central eyespot to nearly uncolored seeds except for black spots on the hilum prominences.

Figures 1 and 2 are photographs of the seed types segregating in two F_2 populations. Both illustrate the three seed-length phenotypes—short, medium, and long—and between them are shown the six color phenotypes used in parents or which segregated from crosses. Figure 1 shows flat black, stippled or dotted black, tan, clump, and white-tan-tip; figure 2 shows tan, red, white-tan-tip, and white-pink-tip.

TABLE 1.—*Varieties of watermelon and phenotypes used in studying inheritance of seed-coat color and seed length*

Seed-coat color	Distribution of varieties having indicated approximate range in seed length		
	Short, 4.5–7.5 mm.	Medium, 7.5–11.5 mm.	Long, 11.5–16.5 mm.
Black		Klondike Winter Queen	Hope Giant.
Tan	Baby Delight		Thurmond Grey.
Clump		Striped Klondike	Golden Honey. Sun Moon and Stars. Northern Sweet.
White-tan-tip			Chilean. Gandy. Sun Moon and Stars. Peerless. Pride of Muscatine.
White-pink-tip			Peerless.

Phenotypes of watermelon seed-coat color are sometimes difficult to classify because of variable degrees of expression. The ordinary fluctuation may be accentuated by other factors, such as stage of

maturity (especially fermentation) and sunburn either on whole or on broken fruits. A customarily easily classified color, such as red or white-tan-tip, is rendered difficult to classify in populations that rotted before seed collection.

Classification of seed-coat color requires time and patience to match and rematch samples from different fruits, and it is best done in the laboratory from cleaned and dried material.

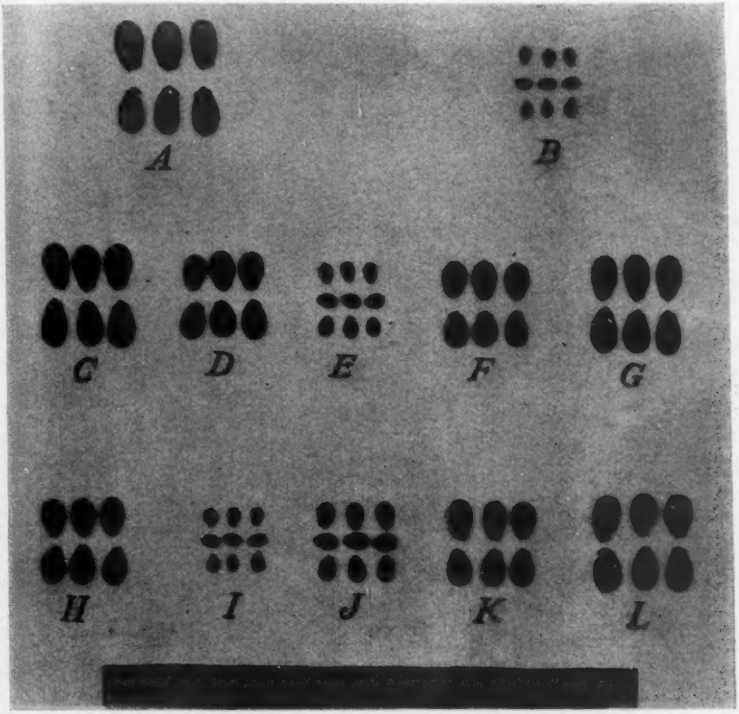

FIGURE 1.—Seed phenotypes segregating in an F_2 population: *A*, Clump long; *B*, tan short; *C*, flat black long; *D*, flat black medium; *E*, flat black short; *F*, stippled or dotted black medium; *G*, dotted black long; *H*, tan medium; *I*, tan short; *J*, clump short; *K*, clump medium; *L*, white-tan-tip long. For phenotypic ratio, see figure 13.

Seed samples were collected from each fruit and were stored in envelopes with cellophane windows (fig. 3). The measurements of 5 or 10 seeds, depending on the uniformity of the sample, were then recorded on the outside of the envelope. The measurements were made with a vernier caliper to tenths of a millimeter, and the genetic analysis was based on the nature of the frequency distribution obtained. The envelopes of an entire population may be spread on the floor or on a large table, as in figure 4, to present a simultaneous view of the seed-coat color and length and to facilitate the necessary color matching.

All Mendelian ratios were tested for goodness of fit by orthogonalized chi-square formulas developed by Fisher (*1*) and applied extensively by Mather (*6*). This sensitive treatment of the standard method disassembles chi-square values into the component values contributed by each degree of freedom present.

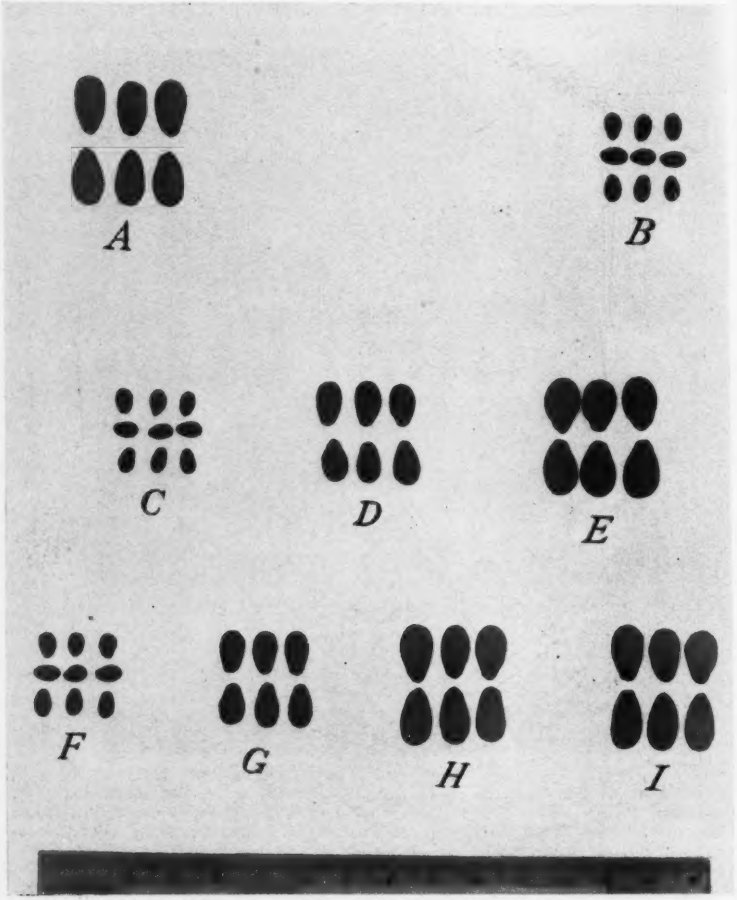

FIGURE 2.—Seed phenotypes segregating in an F_2 population: *A*, White-pink-tip long; *B*, tan short; *C*, tan short; *D*, tan medium; *E*, tan long; *F*, red short; *G*, red medium; *H*, white-tan-tip long; *I*, white-pink-tip long. For phenotypic ratio, see figure 12.

When statistical analysis required grouping for the study of inheritance of quantitative characters, such grouping was based on the size of a significant difference. Frequency tables and frequency polygons drawn from the writers' data are consequently highly useful in interpreting the Mendelian phenomena involved. Graphic treatment in frequency polygons made from seed-length data shows the

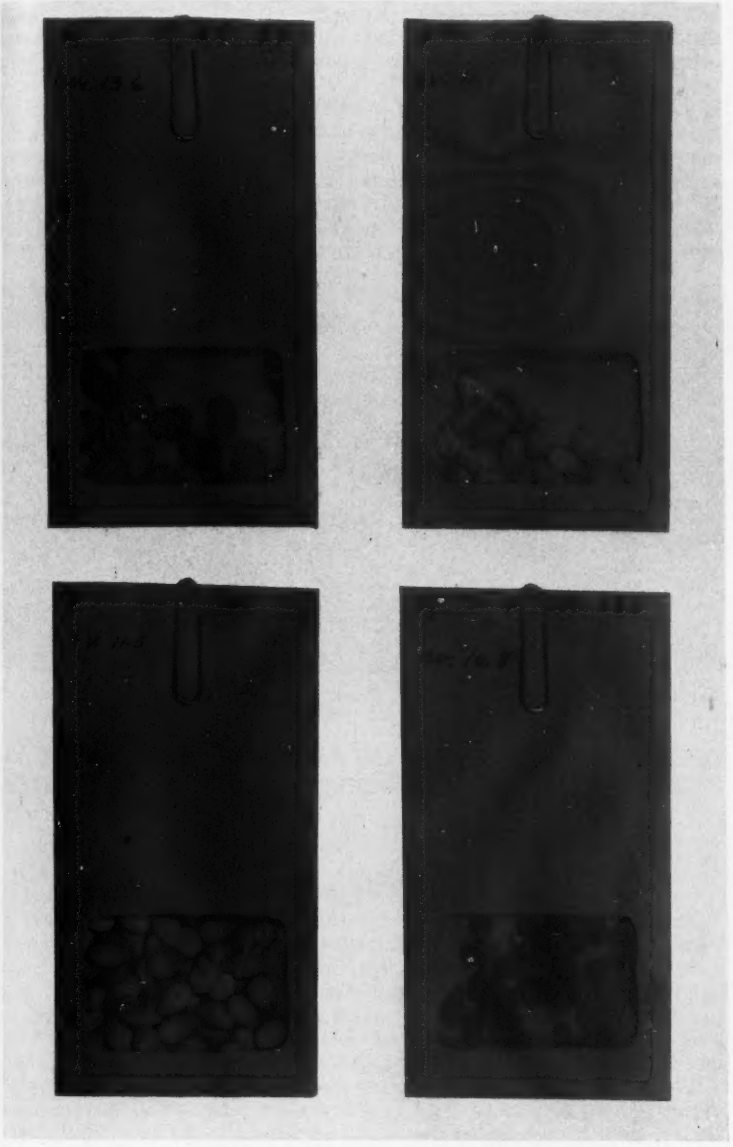

FIGURE 3.—Type of envelope with cellophane window used for storing seed samples to be classified for seed-coat color.

Mendelian proportions as sharply differentiated groupings, and little difficulty exists in distinguishing one phenotype from another, except occasionally with regard to the separation of medium from long in seed-length studies.

Linkage intensities from F_2 data were estimated by two methods of Fisher (*1*), T_3 and T_4, better known as the product method and the maximum-likelihood method, respectively. The product method is the most useful in its dependability and ease of manipulation if solved by Immer's solution, which requires the use of tables for the linkage values as well as the standard errors.[3] The maximum-likelihood method is needed if any phenotype in the observed ratio con-

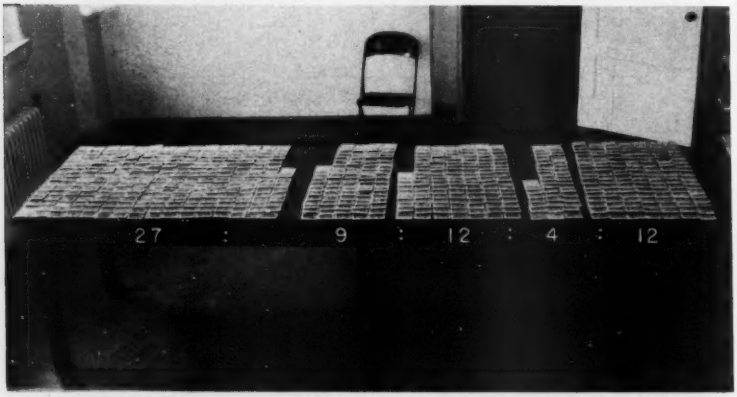

FIGURE 4.—Envelopes of an entire F_2 population segregating in the ratio of 27 : 9 : 12 : 4 : 12 seed-coat color phenotypes.

tains fewer than 10 plants. The formula used for calculating the linkage value by maximum likelihood is that supplied by Owen (*7*), viz,

$$p^2=\frac{a-2(b+c)-d+\sqrt{(a-2(b+c)-d)^2+8dn}}{2n}.$$

INHERITANCE OF SEED LENGTH

An F_2 population of 83 plants from the cross Sun Moon and Stars × Winter Queen, when analyzed for width and length of seeds, disclosed a coefficient of correlation, $r=0.913$, between length and width. Since length of seed is the more variable character and with respect to size presents the same kind of information as width, it was adopted for the analysis of genetic factors for seed size and for the investigation of the interactions with seed-coat color factors.

This reasoning should be sound in the present study because all varieties used as parents had long narrow seeds, but it is recognized that if any broad-seeded varieties (as found in some citron melon types) had been used, then length alone might have been misleading.

Data for seed length were available in crosses involving the combinations 6 mm. × 13 mm., 6 mm. × 10 mm., and 10 mm. × 13 mm. The three lengths 6 mm. (short), 10 mm. (medium), and 13 mm.

[3] Dr. Immer has made available for mimeographed distribution by the Association of Southern Agricultural Workers a revision of his tables with standard errors to replace the probable errors shown in the original (*2*).

(long) represent approximate mean lengths and about the modal class in frequency distributions in segregating populations. Figures 1 and 2 show the three seed lengths segregating in two F_2 populations. Seed from a fruit of a given pure line of medium length may exhibit a larger or smaller mean length than is indicated. For instance, in Klondike, a variety of medium seed length, the seeds have an average length of 9.0 mm., yet F_1 and F_2 populations segregating from crosses to races having shorter or longer seeds usually show the mode of the medium group at approximately 10 mm., whereas the mode for this size group in backcrosses to a shorter or longer parent will be shifted in the direction of the parent's seed length. This situation is also found for long seed length, where individual inbred lines measuring 12.8 or 13.8 mm., when crossed with other seed lengths, have group modes at approximately 13 mm. in the F_2 generations, but less in backcrosses to short-length seed.

Data on observed phenotypic ratios from 17 F_2 and 19 backcross populations analyzed in this study of seed length are summarized in tables 2 and 3, and data on the 43 F_3 populations are summarized in table 4.

TABLE 2.—*Summary of F_2 and backcross generation data from four types of crosses involving seed length*

Type of cross	Phenotypes				Chi-square value[1] due to indicated gene		
	Medium (*LS*)	Long (*ls*)	Short (*Ls*+*ls*)	Total	χ^2_L	χ^2_S	χ^2_{total}
	Number	*Number*	*Number*	*Number*			
(1) F_2 medium × long	1,325	431		1,756	0.171		
(a) F_1 × long	312	301		613	.163		
(b) F_1 × medium	442			442	0		
(2) F_2 medium × short	205		77	282		0.681	
(a) F_1 × short	105		95	200		.405	
(b) F_1 × medium	172			172		0	
(3) F_2 long × short	1,067	309	411	1,787	4.613*	3.708	8.591*
(a) F_1 × long	98	80		178	1.624		
(b) F_1 × short	302		259	561		3.144	
(4) F_2 long × long		542		542	0		
(a) F_1 × long		252		252	0		

[1] A single asterisk (*) indicates significant deviation at the 19 : 1 level for the ratio $3L : 1l$.

TABLE 3.—*Analysis of seed-length data from F_2 populations from the cross short (ssLL) × long (SSll), segregating approximately 9 medium to 3 long to 4 short*

F_2 population	Phenotypes				Chi-square value[1] due to indicated gene, and corresponding degrees of freedom			
	Medium (*LS*)	Long (*ls*)	Short (*Ls*+*ls*)	Total	χ^2_L	Degrees of freedom	χ^2_S	Degrees of freedom
	Number	*Number*	*Number*	*Number*				
A	147	45	33	225	0.250	1	12.813**	1
B	56	31	22	109	5.245*	1	1.349	1
C	20	12	6	38	2.667	1	1.719	1
D	79	12	32	123	6.773**	1	.068	1
E	42	2	7	51	9.818**	1	3.458	1
F	139	45	53	237	.029	1	.878	1
G	317	91	141	549	1.441	1	.103	1
H	151	39	67	257	1.796	1	.105	1
I	116	32	50	198	.730	1	.000	1
Total	1,067	309	411	1,787	28.749**	9	20.493*	9
Deviation					4.613*	1	3.708	1
Heterogeneity					24.136**	8	16.785*	8

[1] A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 4.—*Summary of ratios obtained in the F_3 generation of 43 populations from the cross long (lS) × short (Ls), Peerless × Baby Delight*

Parentage in F_2	Segregation ratio in F_2	Observed frequency	Calculated frequency	χ^2 value	χ^2 value for 19:1 significance
Medium (*LlSs*)	9 medium to 3 long to 4 short.	13/26	11.6/26		
Medium (*LLSs*)	3 medium to 1 short	4/26	5.8/26		
Medium (*LlSS*)	3 medium to 1 long	6/26	5.8/26		
Medium (*LLSS*)	All medium	3/26	2.9/26	0.361	7.815
Long (*llSs*)	3 long to 1 short	5/12	8/12		
Long (*llSS*)	All long	7/12	4/12	3.375	3.841
Short (*LLss+llss+Llss*)	All short	5/5	5/5	0	
Total populations		43	43.1		

Table 2 clearly shows that when medium is crossed with either long or short the F_2 and backcross ratios indicate monohybrid control of seed length. The presence of the two pairs of genes *L* vs. *l* (medium vs. long) and *S* vs. *s* (medium vs. short) is confirmed by the ratios obtained from the cross long × short. The summarized F_2 populations in the latter cross offer the only instance of unsatisfactory fit between the observed and calculated proportions based on the Mendelian ratio 9 medium to 3 long to 4 short. However, backcrosses to both parents fulfill expectations.

The difficulty with the summarized F_2 of long × short is somewhat resolved by a study of the nine individual populations which comprise the summary. Table 3 shows all phenotypic ratios, together with the chi-square values traceable to each degree of freedom: χ^2_s for separation of *S* from *s*, and χ^2_l for separation of *L* from *l*. Except for population A (table 3), all the populations show expected proportions of 3 *S* to 1 *s*. In populations B, D, and E are three exceptions to the majority evidence favoring proportions of 3 *L* to 1 *l*. Examination of the frequency polygons in figures 5, 9, and 10 explains the difficulty of classifying medium (*L*) and long (*l*). Figure 5, especially, shows the distance separating the group modes in parents and F_2 to be greater between short and medium than between medium and long. The long end of the medium distribution overlaps the medium end of the long distribution, hence the difficutly of precisely separating the two phenotypes.

An F_3 generation from the cross long × short (Peerless × Baby Delight) comprised 43 populations and covered about 20 acres. Table 4 displays the seed-length data for the entire F_3 generation summarized as to F_2 parentage, the type of segregation found, and the correspondence between observed and calculated frequencies of parental genotypes for all 43 populations. The presence of five F_3 populations segregating 3 long to 1 short, together with the fact that all five short-derived populations bred true, proves that the double-recessive phenotype is short, a fact which could only be inferred from F_2 data.

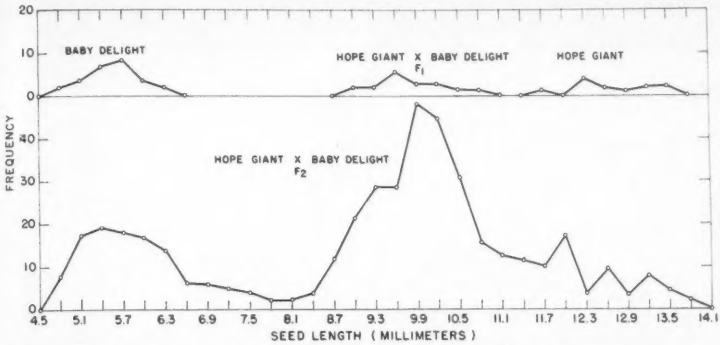
BABY DELIGHT
HOPE GIANT X BABY DELIGHT F1
HOPE GIANT
HOPE GIANT X BABY DELIGHT F2
FREQUENCY
SEED LENGTH (MILLIMETERS)
4.5 5.1 5.7 6.3 6.9 7.5 8.1 8.7 9.3 9.9 10.5 11.1 11.7 12.3 12.9 13.5 14.1

FIGURE 5.—Frequency polygons drawn from the seed-length data for Baby Delight, Hope Giant, and the F_1 and F_2 generations of the cross Hope Giant (long) × Baby Delight (short). Note the practical absence of overlap between short and medium as compared with the amount present between medium and long. The situation in F_2 parallels that existing between the parents and F_1.

In conclusion, the following gene symbols and their interaction effects as encountered in the foregoing investigation are proposed:

Medium, 10 mm., *LS*, two dominants with complementary interaction.
Long, 13 mm., *lS*, the differential effect of *l* produces long.
Short, 6 mm., *Ls*, *ls*, the differential effect of *s* produces short length in both the single- and double-recessive phenotypes.

INHERITANCE OF SEED-COAT COLOR

Investigation of seed-coat color discloses a more complex situation than that of seed size, as is expected with a larger number of phenotypes available. Phenotypic ratios from classification of 20 F_2 and 20 backcross populations are shown in tables 5, 6, 7, and 8.

The data of table 5 indicate that probably black is dominant to all other colors and patterns studied; the relationship is a single-factor dominance when black is crossed with tan or clump, but it is a two-factor dominance when black is crossed with white-tan-tip. When tan is crossed with white-tan-tip, tan is dominant by a single-factor difference; in the cross tan × white-pink-tip, tan is dominant by two factors; but when tan is crossed with clump, black is synthesized in F_1 and there is a dihybrid segregation in F_2 of 9 black to 3 tan to 3 clump to 1 white-tan-tip. When clump is crossed with white-tan-tip there is a monohybrid segregation in F_2, and when a light tan, as in Baby Delight, is crossed with darker tan, as in Thurmond Grey, there is the same color range in F_2 as in each segregating phenotype designated above as tan. The analysis will be simplified as much as possible by using the following working gene hypothesis for the genetic interactions, and by adopting the initial characterizing the first recessive gene found, according to the practice followed in investigations on *Drosophila* and *Zea mays*.

TABLE 5.—*Summary of F_2 and backcross generation data obtained from 8 types of seed-coat color crosses*

Type of cross	Phenotypes							Chi-square value [1] due to indicated gene				
	Black (*RTW*)	Tan (*RtW*)	Clump (*RTw*)	Red (*rtW*)	White-tan-tip (*Rtw*)	White-pink-tip (*rtw*)	Total	χ^2_t	χ^2_w	χ^2_{t+w}	χ^2_r	χ^2_{r+w}
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*					
(1) F_2 black × tan	528	180					708	0.047				
(a) F_1 × black	289						289	0				
(b) F_1 × tan	162	154					316	.155				
(2) F_2 black × clump	309		98				407		0.138			
(a) F_1 × black	214						214		0			
(b) F_1 × clump	146		144				290		.003			
(3) F_2 black × white-tan-tip	299	115	101		29		544	.551	.297	1.307		
(a) F_1 × black	110						110			0		
(b) F_1 × white-tan-tip	14	18	33		15		80	2.112	2.813	5.513*		
(4) F_2 tan × clump	623	231	234		45		1,133	.214	.066	12.639**		
(a) F_1 × tan	59	71					130			.931		
(5) F_2 tan × white-tan-tip		215			53		268		3.627			
(a) F_1 × tan		254					254		0			
(b) F_1 × white-tan-tip		22			23		45		0			
(6) F_2 tan × white-pink-tip		277		96	65	18	456		10.880**		0	0.316
(a) F_1 × tan		481					481					0
(b) F_1 × white-pink-tip		46		23	43	32	144		.174		7.563**	.840
(7) F_2 tan × tan		51					51	0				
(a) F_1 × tan		67					67	0				
(b) F_1 × tan		149					149	0				
(8) F_2 white-tan-tip × clump			410		132		542	.089				
(a) F_1 × white-tan-tip			55		50		105	.152				
(b) F_1 × clump			147				147	.000				

[1] 1 degree of freedom for each. A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 6.—*Phenotypic ratios and chi-square analysis for the 4 F_2 populations from tan × clump seed-coat color crosses*

Population and cross [1]	Phenotypes						Chi-square value [2] due to indicated gene, and corresponding degrees of freedom							
	(*DTW*) black flat	(*dTW*) black stippled	(*tW*) tan	(*Tw*) clump	(*tw*) white-tan-tip	Total	χ^2_d	Degrees of freedom	χ^2_t	Degrees of freedom	χ^2_w	Degrees of freedom	χ^2_{t+w}	Degrees of freedom
	No.	*No.*	*No.*	*No.*	*No.*	*No.*								
1937–a	93	39	37	43	7	219	1.222	1	2.554	1	0.439	1	0.695	1
1938–a	42	19	35	28	3	127	.894	1	1.388	1	.003	1	7.567**	1
1938–b	92	27	49	56	13	237	.137	1	.114	1	1.925	1	2.363	1
1939–b	233	78	110	107	22	550	.000	1	.242	1	.621	1	3.627	1
Total	460	163	231	234	45	1,133	2.253	4	4.298	4	2.988	4	14.252**	4
Deviation							.390	1	.214	1	.066	1	12.639**	1
Heterogeneity							1.863	3	4.084	3	2.922	3	1.613	

[1] a=Thurmond Grey × Striped Klondike; b=Golden Honey × Baby Delight.
[2] Double asterisks (**) indicate significant deviation at 99 : 1 level.

TABLE 7.—*Phenotypic ratios and chi-square analysis for 3 F_2 populations from the cross white-pink-tip × tan (Peerless × Baby Delight) and 2 backcross populations from F_1 × white-pink-tip*

Cross or backcross and population	Phenotypes [1]					Chi-square value [2] due to indicated gene, and corresponding degrees of freedom					
	Tan (*RW*)	White-tan-tip (*Rw*)	Red (*rW*)	White-pink-tip (*rw*)	Total	χ^2_T	Degrees of freedom	χ^2_w	Degrees of freedom	χ^2_{T-w}	Degrees of freedom
White-pink-tip × tan (F_2):	*Number*	*Number*	*Number*	*Number*	*Number*						
1937–a	136	39	43	7	225	0.784	1	2.253	1	0.751	1
1937–b	76	10	35	3	124	1.817	1	13.172**	1	.516	1
1938	65	16	18	8	107	.000	1	.252	1	.757	1
Total	277	65	96	18	456	2.604	3	15.677**	3	2.024	3
Deviation						.000	1	10.880**	1	.316	1
Heterogeneity						2.604	2	4.797	2	1.708	2
F_1 × white-pink-tip:											
1937	23 (3)	26 (3)	12 (1)	6 (1)	67	.045	1	.015	1	1.796	1
1938	23 (1)	17 (1)	11 (1)	26 (1)	77	.052	1	.636	1	4.688*	1

[1] Expected ratio shown in parentheses.
[2] A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 8.—*Summary of data on classification for seed-coat color obtained in the F_3 generation of 43 populations from the cross white-pink-tip (rtw) × tan (RtW)*

Parentage in F_2	Segregation ratio in F_3 [1]	Observed frequency	Calculated frequency	χ^2 value	χ^2 value for 19:1 significance
Tan (*RrWw*)	9 tan to 3 red to 3 white-tan-tip to 1 white-pink-tip.	14/29	12.889		
Tan (*RrWW*)	3 tan to 1 red	6/29	6.444		
Tan (*RRWw*)	3 tan to 1 white-tan-tip	3/29	6.444		
Tan (*RRWW*)	All tan	6/29	3.222	2.775	7.815
Red (*rrWw*)	3 red to 1 white-pink-tip	3/5	3.334		
Red (*rrWW*)	All red	2/5	1.667	0	
White-tan-tip (*Rrww*)	3 White-tan-tip to 1 white-pink-tip.	3/6	4		
White-tan-tip (*RRww*)	All white-tan-tip	3/6	2	.188	3.841
White-pink-tip (*rrww*)	All white-pink-tip	3	3	0	0
Total		43	43.000		

[1] None of the 43 populations involved here showed, by the χ^2 test, a significant deviation from its indicated ratio.

The following genes and phenotype symbols are proposed:

RTWD, black, flat (Winter Queen).

RTWd, *d* differentiates a stippled surface of black dots sometimes numerous enough to be almost solid black; usually, however, a tannish or reddish undercoat is visible in segregating phenotypes; *d* is apparently a specific modifier of black in the cases here considered (Klondike and Hope Giant).

RtW, *t* differentiates tan, which ranges in segregating populations from dark Tuscany brown (*5, pl. 7, L 11*)[4] through cacao (*5, pl. 5, B 11*) as found in

[4] Plate and block numbers of colors given by Maerz and Paul (*5*).

the variety Thurmond Grey down to light leghorn (*5, pl. 10, D 3*) as in the variety Baby Delight.

RTw, w differentiates a pattern called "clump" because of the general tendency for the pigment in seeds of most members of this phenotype in segregating populations to clump together about the margin or in the center. In segregating populations the range of clumping runs at one extreme from uniform distribution of pigment except for a narrow line inside the margin on both surfaces, as in the Sun Moon and Stars variety (fig. 6, *B, a*), to the other extreme of an almost unpigmented seed except for a penciled rim or merely two dots on the hilum prominences, as in the varieties Golden Honey (fig. 6, *A, a*) and Northern Sweet.

Rtw, w and *t* together differentiate a more or less whitish seed called white-tan-tip, sometimes with a slight tannish shading on the margin, but the shading is always present at the hilum end (Pride of Muscatine, Gandy, Chilean, and some lines of Peerless).

rtW, r and *t* together differentiate a red- or reddish-orange colored seed ranging in segregating populations from fez red (*5, pl. 5, L 4*) to powder pink (*5, pl. 3, E 7*). (Not used as a parent but segregating from Peerless × Baby Delight, *rtw*×*RtW*.)

rtw, r, t, and *w* together produce a decidedly white seed with slight pink color at the hilum end, called white-pink-tip, and sometimes a trace of pink along the margin in segregating populations (Peerless).

Table 5 shows that with four exceptions the observed phenotypic ratios conform to expectation based on the foregoing hypothesis of three genes for color. The right-hand side of the table shows the portion of chi square that is contributed by each degree of freedom. The four poor fits are thus traced to the gene interactions responsible.

The first poor fit, black×white-tan-tip backcrossed to white-tan-tip, is due to the interaction between *T* and *W*, although the difficulty in this backcross is peculiar to it because the χ^2_{tw} from the F_2 generation of the same cross shows no disturbance at this point. The second poor fit, in the F_2 of tan×clump, is also due to the interaction of *T* and *W*. In table 6 the four populations resulting from this cross are orthogonalized separately, and show that the difficulty between *T* and *W* is peculiar to the one population of Thurmond Grey× Striped Klondike grown in 1938. The three other populations segregate according to hypothesis. Hence it may be concluded that no genetic linkage occurs between the genes *T* and *W*.

The third and fourth cases of unsatisfactory fit occur in the F_2 progenies of tan×white-pink-tip and the backcross of this F_1 to white-pink-tip. In the F_2 populations χ^2_w is involved; in the backcross χ^2_r is involved. Table 7 shows that the 1937–a population was a single F_2 generation of 225 plants, whereas the 1937–b population was an aggregate of three smaller F_2 populations of 25, 73, and 26 plants, respectively. The failure of the combined three populations to give an acceptable χ^2_w when totaled is ascribed to the inadequate sizes of the populations individually and collectively. (N. B.—The seed-length gene *L* has already been seen in the foregoing section to be irregular in this same cross.) In the first backcross population it was assumed that F_1 was heterozygous for the *r* allelomorphs; hence a 3:3:1:1 ratio in this backcross gives a satisfactory χ^2 estimate. In the second backcross population an excessive proportion of white-pink-tip at the expense of red is responsible for the disturbed interaction between the *r* and *w* genes.

The cross white-pink-tip × tan (Peerless × Baby Delight) recovers the parental phenotypes as double recessive and double dominant, respectively, in a dihybrid segregation, and the two single recessives are white-tan-tip and red. When one of these single recessives,

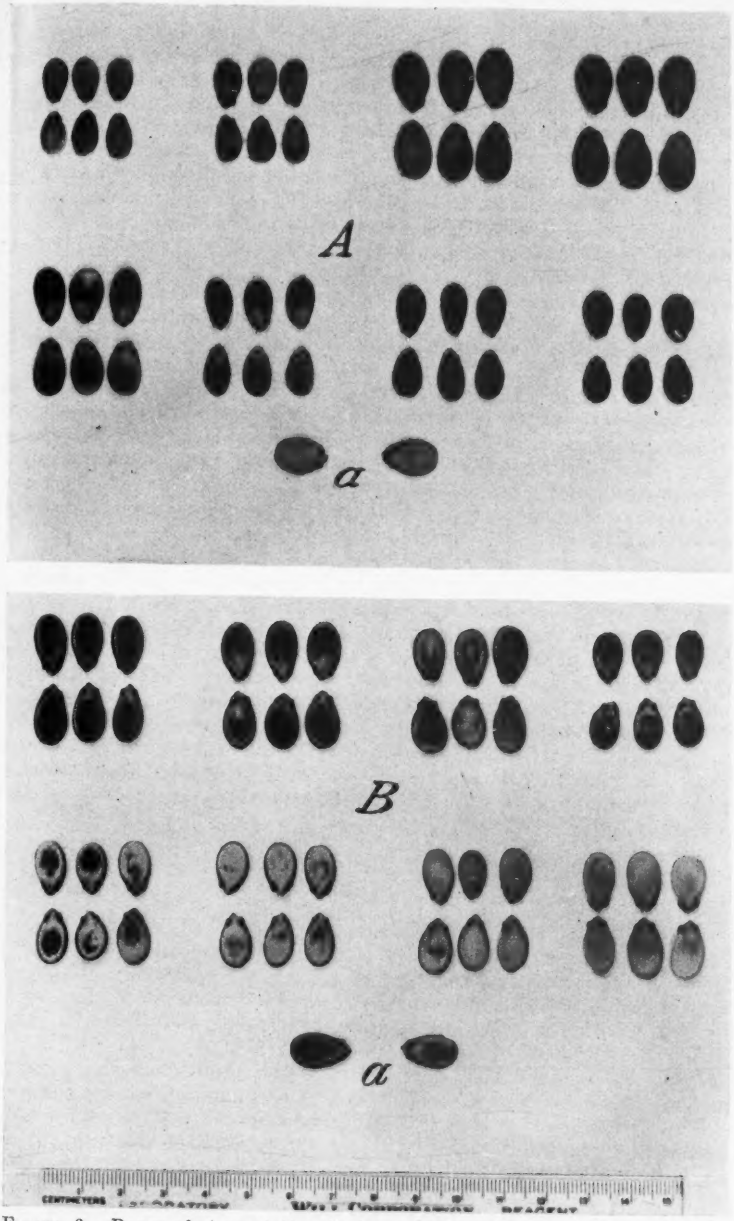

FIGURE 6.—Range of pigmentation displayed in clump phenotype when segregating in the F_2 populations of two crosses. *A*, Golden Honey (restricted clump) × Baby Delight (tan); *a*, sample from clump parent. *B*, Sun Moon and Stars (full clump) × Klondike (black); *a*, sample from clump parent.

white-tan-tip, is crossed with black (Pride of Muscatine × Klondike), the two parental forms are also recovered as double recessive and double dominant, respectively, while the two single recessives are tan and clump. The inference arises that in these two crosses, instead of four different genes, there are only three. All other crosses in table 5 confirm this hypothesis. Accordingly, the three genes *RTW* are postulated as the chief elements in a gene-interaction system in which simultaneous segregation of *R* and *T* has not yet been obtained, while *RW* and *TW* are shown to be inherited independently of each other. A fourth gene, *D* (table 6), operates as a modifier of black, *RTW*, and the simple allelic relations of *D* and *d* are demonstrated by four separate F_2 populations.

In Golden Honey the clump background has almost disappeared except for a marginal band and shading on the hilum end as shown in figure 6, *A*, *a*. There is no question that the recessive allel *w* is present with *T*, and that when it is crossed with tan in Golden Honey × Baby Delight the same full range of the pattern is displayed in three-sixteenths of the F_2 population as in the two other crosses, Sun Moon and Stars (white-tan-tip) × Klondike (black) and Thurmond Grey (tan) × Striped Klondike (clump). Figure 6 shows the range of expression of the phenotype clump in F_2 populations of the crosses (*A*) Golden Honey × Baby Delight and (*B*) Sun Moon and Stars (white-tan-tip race) × Klondike (black).

In order to test further the validity of the color-genes hypothesis adopted from the foregoing F_2 and backcross studies, an F_3 generation from the cross white-pink-tip × tan was grown in the summer of 1939. A summary of the data obtained from F_3 classification for seed-coat color appears in table 8, and is arranged to show F_2 parentage, the type of segregation found, and the correspondence between observed and calculated frequencies. Out of 29 populations derived from tan-colored parents, 6 proved to be from *RRWW* and 3 from *RRWw* parents, thus constituting a slight but not serious reversal of expectation; the remaining 34 populations shown in the table segregated in close agreement with the Mendelian proportions expected from the foregoing hypothesis.

RELATION BETWEEN SEED-LENGTH AND SEED-COAT COLOR GENES

Figures 7 and 8 show that when the allels *T* (black) and *t* (tan) interact with *L* and *l* (medium and long) or *S* and *s* (medium and short) there is independent assortment of all genes concerned. The two figures graphically demonstrate the complete dominance of black over tan, medium over large, and medium over small, as well as the independent assortment of the three pairs of genes into two 9 : 3 : 3 : 1 ratios in F_2, 1 : 1 : 1 : 1 in backcrosses to the double-recessive parent, and all black medium in backcrosses to the triple-dominant parent.

The first indication that linkage existed between quantitative and qualitative genes for seed characters arose from the observation in 1935 that small white seeds were rare in crosses expected to produce them. The approximate linkage value calculated from the 1935 cross of large white-pink-tip × small tan (Peerless × Baby Delight) was also obtained in 1937 from the cross large white-tan-tip × medium

black (Pride of Muscatine × Klondike). The observation that white-tan-tip was involved in linkage with white-pink-tip in the first cross and that white-tan-tip and clump were involved in the other

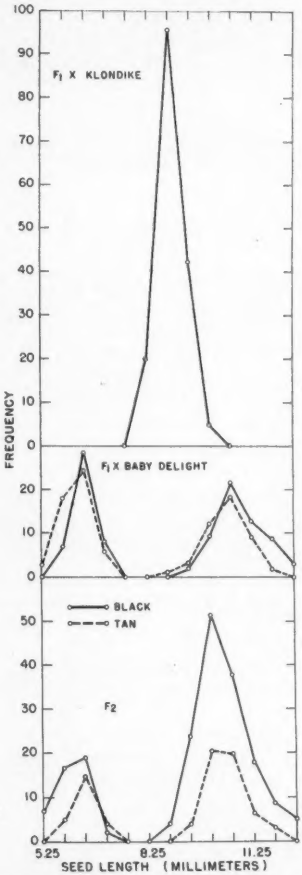

FIGURE 7.—Klondike × Baby Delight (black medium × tan short): F_2 and backcrosses to both parents, segregating for two color and two length phenotypes—black, tan, medium, and short.

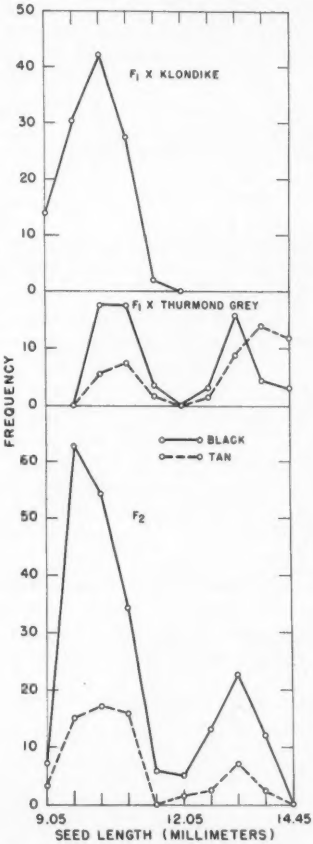

FIGURE 8.—Klondike × Thurmond Grey (black medium × tan long): F_2 and backcrosses to both parents, segregating for two color and two length phenotypes—black, tan, medium, and long. Independent assortment.

cross suggested that the same recessive gene *w* helps determine the two white phenotypes and clump.

In the study of the interactions of the four color and two seed-length genes by orthogonal chi-square values, no population available included at one time more than four of the six. The two crosses offer-

ing quadrihybrid segregations (table 9) were Golden Honey × Baby Delight, segregating for *LST* and *W*, and Peerless × Baby Delight segregating for *LSR* and *W* (see figs. 12 and 13). Several crosses segregated for *LT* and *W*, but they will not be discussed since they add nothing to the information from table 9 or from previous tables. Since the allels *D* and *d* operate only on the black phenotype, gene interaction is limited to *D* and *L* or *D* and *S*, the effects of which are seen in tables 10 and 11.

TABLE 9.—*Sources of chi-square from the 9 possible single and double genic interactions in the 2 F_2 populations Golden Honey × Baby Delight (lSTw × LstW) and Peerless × Baby Delight (lSrw × LsRW)*

Golden Honey × Baby Delight			Peerless × Baby Delight		
Phenotypes		Chi-square value [1]	Phenotypes		Chi-square value [1]
Gene symbol	Number		Gene symbol	Number	
LSTW	293	$\chi^2_l = 0.462$	*LSRW*	178	$\chi^2_l = 0.014$
LSTw	48	$\chi^2_s = 0.646$	*LSRw*	18	$\chi^2_s = 6.707^{**}$
LStW	99	$\chi^2_t = 0.004$	*LSrW*	74	$\chi^2_r = 0$
LStw	8	$\chi^2_w = 0.034$	*LSrw*	7	$\chi^2_w = 9.793^{**}$
L+lsTW	91		*L+lsRW*	73	
L+lsTw	47	$\chi^2_{lt} = 0.282$	*L+lsRw*	2	$\chi^2_{lr} = 3.141$
L+lstW	46	$\chi^2_{lw} = 147.260^{**}$	*L+lsrW*	15	$\chi^2_{lw} = 105.816^{**}$
L+lstw	2		*L+lsrw*	0	
lSTW	43		*lSRW*	28	
lSTw	68	$\chi^2_{st} = 0.062$	*lSRw*	45	$\chi^2_{sr} = 2.935$
lStW	14	$\chi^2_{sw} = 0.062$	*lSrW*	5	$\chi^2_{sw} = 9.899^{**}$
lStw	26	$\chi^2_{tw} = 6.065^{*}$	*lSrw*	13	$\chi^2_{rw} = 0.024$
Total	785	$\chi^2 = 154.877^{**}$	Total	458	$\chi^2 = 138.329^{**}$

[1] One degree of freedom for each. A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 10.—*Frequency distribution of phenotypes and chi-square analysis of gene interaction between stippling and long-seed factors in (Hope Giant × Baby Delight) × Hope Giant*

Cross	Phenotypes (all black)					Chi-square value [1] due to indicated gene and corresponding degrees of freedom					
	Flat medium (*DL*)	Flat long (*Dl*)	Stippled medium (*dL*)	Stippled long (*dl*)	Total	χ^2_d	Degrees of freedom	χ^2_l	Degrees of freedom	χ^2_{dl}	Degrees of freedom
	Number	*Number*	*Number*	*Number*	*Number*						
WBC 19a	66	54	74	52	246	0.102	1	4.427*	1	0.260	1
WBC 19c	41	28	28	30	127	.787	1	.787	1	1.543	1
Total	107	82	102	82	373	.889	2	5.214	2	1.803	2
Deviation						.043	1	5.190*	1	.043	1
Heterogeneity						.846	1	.024	1	1.760	1

[1] A single asterisk (*) indicates significant deviation at 19:1 level.

TABLE 11.—*Chi-square analysis of gene interaction between stippling and long- and short-seed factors, using only relevant data from F_2 populations also segregating for phenotypes that are omitted here*

Phenotypes		Chi-square value [1]	Degree of freedom	Phenotypes		Chi-square value [1]	Degree of freedom
Gene symbol	Number			Gene symbol	Number		
DL	237	$\chi^2_D=1.252$	1	*DS*	79	$\chi^2_D=0.003$	1
Dl	57	$\chi^2_L=9.779^{**}$	1	*Ds*	13	$\chi^2_S=12.160^{**}$	1
dL	94	$\chi^2_{DL}=1.089$	1	*dS*	30	$\chi^2_{DS}=1.518$	1
dl	17			*ds*	1		
Total	405	12.120**	3	Total	123	13.681**	3

[1] Double asterisks (**) indicate significant deviation at 99:1 level.

In the Golden Honey × Baby Delight population, out of 12 phenotypes (therefore 11 degrees of freedom), only the 9 single and double gene interaction degrees shown in table 9 are useful. The two triple gene interaction degrees remaining give no linkage information and may be omitted. The data show a strong presumption of linkage ($\chi^2_{LW}=147.260$, 1 degree of freedom) for the genes *L* and *W*, and a weak one for *T* and *W* ($\chi^2_{TW}=6.065$). This last-named value ($\chi^2_{TW}=6.065$) has already been analyzed (table 6) for the two sections of the population grown in 1938 and 1939, respectively. The two chi-square values in question are 2.363 and 3.627, neither of which is significant, although when the two sections are summated to make one population, as in table 8, the individual shortages of white-tan-tip plants accumulate to give an increased chi-square value.

Interaction of *L* and *S* cannot be studied in this or any other cross because in the 9:3:4 ratio present there are but 2 degrees of freedom, viz, *L* vs. *l*, and *S* vs. *s*, and there is no way of orthogonalizing the *L* vs. *S* interaction. However, if *L* and *S* were linked, then *S* and *W* would also be linked, since linkage exists between *L* and *W* (see data to follow).

The Peerless × Baby Delight cross will give little reliable information on the genes *S* and *W* or their interaction, because the single-gene chi-square values are each statistically significant. $\chi^2_{lw}=105.816$ indicates the second strong presumption of coupling linkage between *L* and *W* in corroboration of the preceding cross evidence. A second significant chi square, $\chi^2_{slw}=9.899$, 1 degree of freedom, probably results from that given above. χ^2_{sr} and χ^2_{rw} are nonsignificant; consequently the chances are against the presence of any linkages in this series except the possible but yet untried combination *T* and *R*, and the highly probable *L* and *W*, to be evaluated later.

A graphic portrayal of the different types of factor interaction between these color and length genes is presented in the series of frequency polygons (figs. 7–13). All crosses not represented graphically are in agreement with the interpretations offered.

The gene interactions involving *D* with *L* from the backcross (black stippled long × tan flat short) × black stippled long are shown

in table 10. Data are given for the only two backcross populations where interaction of *D* with *L* can be followed without interference from other genes. Both populations are from the same parentage and both give the information that no linkage relations exist between genes *L* and *D*. No crosses are available showing only the interaction of *D* with *S*, since that would require an as yet unknown parent having stippled black short seeds. However, it is possible to approximate the relations of *D* with *L* and *S* by omitting irrelevant data from more complex F_2 segregations. These approximations are given in table 11 and show that within such limits no linkage relations exist between *D* with either *L* or *S*.

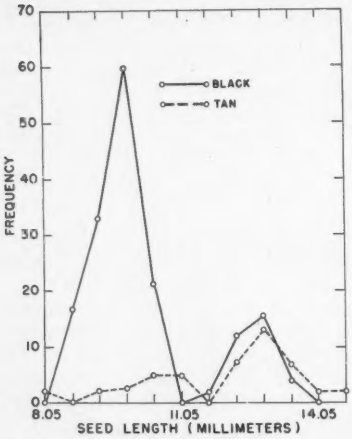

FIGURE 9.—Klondike × Sun Moon and Stars (black medium × clump long): F_2 population showing coupling-phase linkage between *L* and *W*.

The only gene interaction manifesting clear-cut demonstrations of linkage is that between *L* and *W*, the effects of which are graphically shown in figures 9 to 13. In table 12 are given all relevant data on 10 F_2 populations in the coupling phase, 2 F_2 populations in repulsion, and 7 backcrosses, together with estimates of linkage intensity.

The populations in coupling and backcross to the double-recessive parent are always most satisfactory for estimating linkagein tensities, as Fisher (*1*) and Mather (*6*) have shown; consequently the coupling value 19.3 ± 1.1 percent, estimated by the product method, and the value 15.8 ± 1.2 percent, obtained by direct calculation of cross-overs in the backcross, give a more reliable estimate than the value 24.7 ± 4.9 percent, estimated by the maximum-likelihood method from the repulsion populations.

TABLE 12.—*Analysis of sources of chi-square and estimate of linkage value for L and W in populations segregating for seed length (medium, L, vs. large, l), and seed color (black or tan, W, vs. white or clump, w), in 10 F_2 coupling, 2 F_2 repulsion, and 7 backcross populations*

Type of cross	Phenotypes					Linkage value	Chi-square value due to indicated gene [1] [2]		
	LW	*Lw*	*lW*	*lw*	Total		χ^2_L	χ^2_W	χ^2_{LW}
	Number	Number	Number	Number	Number				
Coupling	1,031	124	140	240	1,535	19.3±1.1	0.006	1.355	416.591
Repulsion [3]	195	77	71	4	347	24.7±4.9	1.945	.424	16.089
Backcross	362	74	67	389	892	15.8±1.2	.405	1.221	414.422

[1] Each χ^2 has 1-degree of freedom.
[2] Significance level: 19 : 1=3.841; 99 : 1=6.635.
[3] By maximum-likelihood method.

However, Mather (*6, p. 63*) shows that a completely classified F_2 population "contains twice as much information about the recom-

bination fraction" as a backcross. Consequently, the fact that the 43 F_3 populations indicate the full genotypes of their F_2 parents

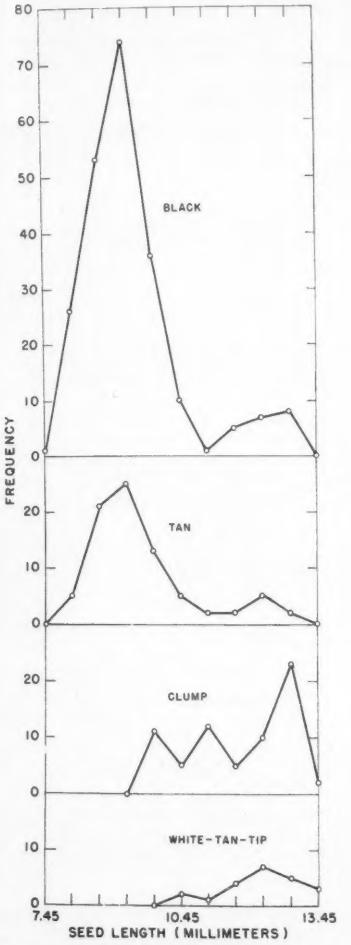

FIGURE 10.—Pride of Muscatine × Klondike (white-tan-tip long × black medium): F_2 population segregating in four color phenotypes, black, tan, clump, and white-tan-tip, and two length phenotypes, medium and long. Coupling linkage between *L* and *W*.

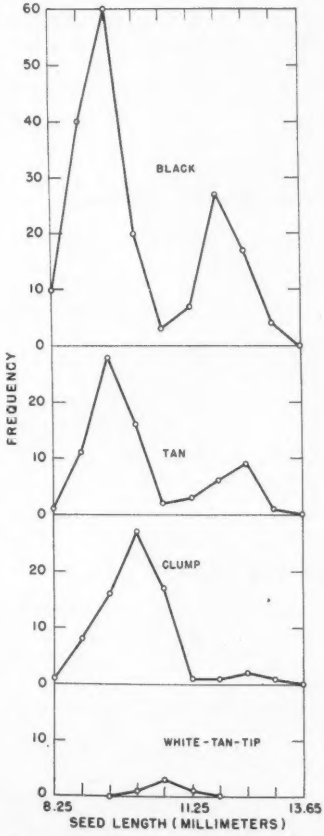

FIGURE 11.—Striped Klondike × Thurmond Grey (clump medium × tan long): F_2 population segregating in same phenotypes as in figure 10, but with repulsion linkage between *L* and *W*.

provides a degree of precision not available in the data of table 12. In table 13, therefore, is seen the distribution by phenotype symbols for *L* and *W* of the 43 parent plants of the F_3 generation. Calculation

of the linkage value by the maximum-likelihood method indicates a coupling linkage value of 21.52±7.24 percent, which falls within the range indicated by the data of table 12.

TABLE 13.—*Analysis of the distribution by phenotype symbols of the identified genotypes of 43 parent plants of the F_3 generation from the cross LW × lw (tan medium × white-pink-tip long)*

Phenotype	Frequency	Chi-square value[1]	Linkage value
			Percent
LW	29	χ^2_L=0.070	21.52±7.24
Lw	2	χ^2_*=0.628	
lW	6	χ^2_{Lw}=6.721**	
lw	6		
Total	43		

[1] Double asterisks (**) indicate significant deviation at 99:1 level.

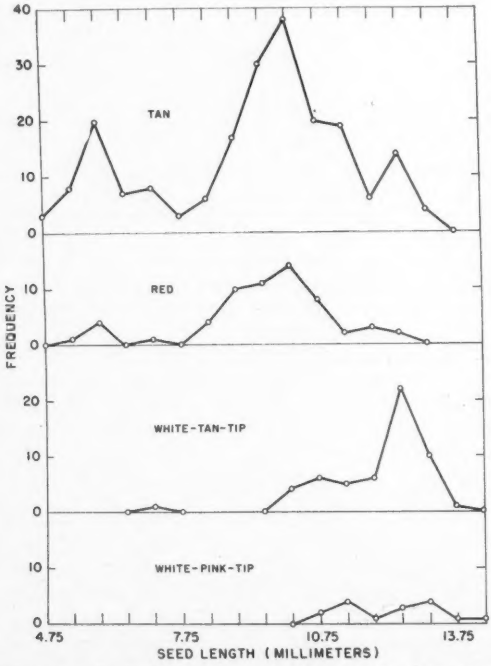

FIGURE 12.—Peerless × Baby Delight (white-pink-tip long × tan short): F_2 population segregating in four color phenotypes, tan, red, white-tan-tip, and white-pink-tip, and three length phenotypes, short, medium, and long. Coupling linkage between *L* and *W*.

DISCUSSION

Weetman (*9*) studied inheritance of seed weight (units of 25 seeds per fruit) of F_1, F_2, and backcross populations for the cross light (1.22±0.01 gm.) × heavy (2.60±0.05 gm.). In reciprocal crosses the

F_1 mode varied a little, but was always closer to the mode of the lighter than the heavier parent. F_2 and backcross distributions were bimodal, suggesting segregations of 3 light to 1 heavy for F_2, all light for backcrosses to the light parent, and 1 light to 1 heavy for backcrosses to the heavy parent. Despite significant deviations between observed and calculated ratios, these data indicate that Weetman had parental races corresponding to the medium and long-seeded phenotypes of the present investigation. The writers obtained good fits between observed and calculated ratios because all phenotypes had long, narrow seeds, hence variation was mainly in length. There

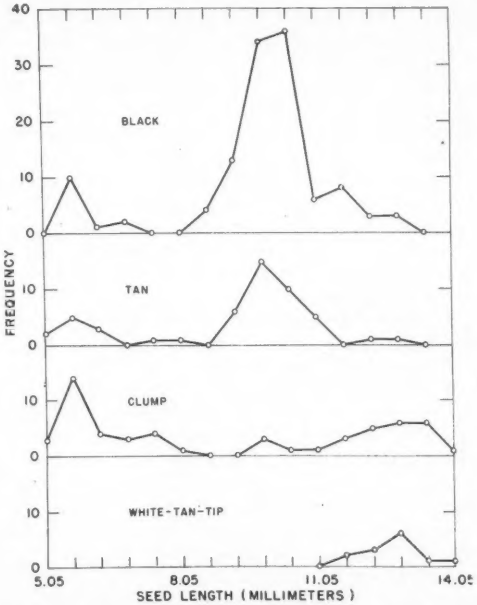

FIGURE 13.—Golden Honey × Baby Delight (clump long × tan short): F_2 population segregating in four color phenotypes, black, tan, clump, and white-tan-tip, and three length phenotypes, short, medium, and long. Coupling linkage between *L* and *W*.

are biotypes in *Citrullus vulgaris*, however, with relatively broad seeds, and crosses between this and the long narrow phenotype would probably segregate for more genes than *L* and *S*. The poor fit observed by Weetman may have arisen from the presence of seed-shape genes in addition to seed-length genes.

In parent varieties there are at least two extreme kinds of clump and tan seed, which, on crossing with another phenotype, present the same shade range in the segregating clump or tan phenotype found in F_2. This shade range within the same phenotype in segregating populations is not caused entirely by accompanying dominant genes of the triple interaction system, for the two original parent varieties Golden Honey and Sun Moon and Stars (fig. 6) were certainly not segregating. The range from light tan identified from Maerz and

Paul (*5*) as leghorn (*pl. 10, D 3*) to dark brown (*Tuscany, pl. 7, L 11*), runs parallel to the shade range of the poultry breed called Brown Leghorn. In the case of poultry the predominant shade is leghorn, as the breed name indicates, but much darker shades of brown are frequently found within the breed, and upon crossing with other phenotypes give the same segregation range as the lighter leghorn shade.

In the early stages of these investigations the extremely reduced clump phenotype of Golden Honey was thought to be white rather than clump (*8*), thus causing some confusion in classifying for color.

Red in segregating populations is another phenotype giving a wide range of shades from fez red to powder pink, as illustrated by Maerz and Paul (*5*), and no doubt different investigators will call this phenotype by different names, such as reddish brown, orange, or red. The recognition of the extent of the shade range comprising a given phenotype is facilitated by using envelopes with cellophane windows when classifying Mendelian populations. For the sake of future genetic studies of watermelon it is important that investigators know they are dealing with a given genotype no matter under what names it may appear. If the range of shading found within a given phenotype is the same from cross to cross and appears repeatedly in the approximate proportions expected by a working hypothesis, then the existence of black, clump, tan, red, white-tan-tip, and white-pink-tip as Mendelian phenotypes is established. Moreover, all the phenotypes but black and clump appear as expected by gene analysis right into F_3, and these fit so adequately into the interaction system here developed, besides confirming the findings of other investigators, as to render further investigations of these two unnecessary.

Kanda (*3*) in 1931 published a preliminary report on inheritance studies in seed-coat color, in which he presented detailed studies of only 2 crosses, one evidently involving the same pair of factors here called black flat and black dotted (*Dd*), which was carried to F_3, and the other postulated as segregating for 4 pairs of factors including *Dd*. About 80 additional crosses reported had not gone beyond F_1. The cross reported to segregate for 4 pairs of factors for seed-coat color comprised 123 plants in F_2 and was carried no further, despite the fact that an adequate analysis required a minimum of 1,024 plants to include the quadruple recessive at 5–percent probability and between 1,590 and 1,855 plants to include it at 1-percent probability. When Kanda's F_2 phenotypic ratio is subjected to a test for goodness of fit, as in table 14, the chi-square value obtained is 12.636, with 5-percent significance at 9.488 and 1-percent significance at 13.277. If his phenotypes are compared with those obtained in the present material, a closer agreement can be reached by assuming only 3 pairs of factors, which will produce a chi-square value of 9.010, with the same levels of significance as given above. In the suggested postulation *T* and *D* are certain (according to the writers' hypothesis) to be included, and *R* is suggested as the third gene. Even if this is an oversimplification, it fits the situations already known, provided Kanda's "brown clear" phenotype comprises the range observed for the writers' "red" phenotype.

Weetman's (*9*) study of seed-color inheritance supports the writers' interpretations when allowance is made for inevitable personal bias in the choice of names for fluctuating shades of color. For example, Weetman's description of Japan 4 seed color as "medium brown with fine black dots" is the writers' dotted black, and his Long Iowa Belle,

described as "light tan with peripheral black band," is the writers' clump (see fig. 6). Description of the F_1 between them as "brown nonbanded * * * much darker, as the fine black dots were more numerous" is an accurate description of the writers' F_1 Thurmond Grey × Striped Klondike, or Golden Honey × Baby Delight. F_2 and the backcross to Long Iowa Belle (clump) gave the expected segregation of 3:1 and 1:1, respectively, and the backcross to Japan 4 (black) was "all brown nonbanded" as expected.

TABLE 14.—*Kanda's F_2 population with gene analysis given, and a proposed revision in the light of the present investigation*

Observed frequency		Kanda analysis		Suggested revision	
Phenotype	Number	Expectation	Postulation	Expectation	Postulation
Black flat	45	38.92	*AMHK*	51.9	*RTD.*
Black dotted	21	13.96	*AMHk*	17.3	*RTd.*
Brown knob	21	17.29	*AMh* (*K* or *k*)	23.1	*rT* (*D* or *d*).
Brown clear	15	23.05	*Am* (*Hh*, *Kk*)	7.7	*rt* (*D* or *d*).
Tan	21	30.75	*aM* (etc.)	23.1	*Rt* (*D* or *d*).
Total	123	122.97		123.1	
χ^2 value [1]		12.636		9.010	

[1] For 5-percent probability=9.488; for 1 percent probability=13.277.

In Weetman's second cross, involving Japan 6 and Long Iowa Belle, the female parent is referred to as "reddish brown" and the F_1 between them as "black," doubtless the writers' flat black. "Reddish brown" in this case is probably the writers' dark tan, which when crossed to clump produced 9 black, 3 "yellowish to reddish brown" (tan), 3 "light tan with black bands" (clump), and 1 "tan quite variable" (white-tan-tip). The backcrosses to both parents segregated as expected for these phenotypes.

In Weetman's third cross, Japan 6 and China 23, the latter had a clump phenotype, and F_2 (expected to segregate 9 : 3 : 3 : 1 as above) was classified as 9 "dark brown or black," 4 "reddish or yellowish brown," and 3 "tan banded." The backcross to the tan parent gave 1 black and 1 tan. In F_2 the 4/16 class was obviously a difficult one to classify and the double-recessive phenotype could not be identified with complete assurance.

The investigations reported by the present writers substantiate the published reports from other sources, and by adding new data coordinate the information now available on form and size of seed and on seven seed-coat color phenotypes into a unified system somewhat resembling the classic system of aleurone colors occurring in *Zea mays* L.

SUMMARY

The three seed-length phenotypes used in this study—short (average about 6 mm.), medium (average about 10 mm.), and long (average about 13 mm.)—behave with respect to each other as though belonging in a dihybrid F_2 segregation of 9 medium (*LS*) to 3 long (*lS*) to 4 short (*Ls* and *ls*).

Seed-coat color phenotypes of parent biotypes used in this study are black, clump, tan, white-tan-tip, and white-pink-tip. Of 10

possible combinations 7 have been made. Analysis of F_2, F_3, and backcross populations indicates that the colors and patterns of this series are determined by the interaction of a system of 3 main genes *RTW* and 1 specific modifier *D* operating on black alone. Of the 8 possible phenotypes from the 3 main genes *RTW*, 6 are indicated, *RTW* (black), *RT*w (clump), *RtW* (tan), *Rtw* (white-tan-tip), *rtW* (red), and *rtw* (white-pink-tip). *RTWd* restricts the black pigment to dots more or less uniformly distributed.

There is no evidence of linkage within groups of the length (*LS*) or color (*DRTW*) genes, since all possible combinations have been analyzed except *TR*. But between groups, the genes *L* and *W* display coupling linkage of 19.3 ± 1.1 in F_2 and 15.8 ± 1.2 in backcross, 24.7 ± 4.9 repulsion linkage in F_2, and 21.52 ± 7.24 coupling linkage among the completely identified parents of an F_3 generation.

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INCREASED SIZE AND NICOTINE PRODUCTION IN SELECTIONS FROM INTRASPECIFIC HYBRIDS OF NICOTIANA RUSTICA [1]

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INTRODUCTION

At present nicotine is obtained from leaf stems and low-grade or scrap-leaf material of *Nicotiana tabacum* L. as a byproduct of the tobacco industry. However, with the possibility of an increasing demand for nicotine, because of its wider use in insecticides and for other purposes, there may be need for varieties of tobacco that can be grown profitably for the nicotine alone.

As early as 1911 *Nicotiana rustica* L. was shown [2] to have a higher content of nicotine than *N. tabacum*, and it was suggested at that time as a logical species to use as a source of this alkaloid.

For a number of years many different varieties and introduced strains of *Nicotiana rustica* have been grown by the Division of Tobacco Investigations and tested for yield and alkaloidal content. Types with the highest yields of nicotine were selected and continued each season. It was considered, however, that even among the best of these there was need for improvement of plant characteristics and especially for larger size.

The late Otto Olson, of this Division, working in cooperation with the Pennsylvania Agricultural Experiment Station, crossed two favorable varieties of *Nicotiana rustica* some years ago, and selections from his hybrids have also been continued.

With these sources of material the investigations reported herein were begun in 1936. Their purpose was to combine the best size characteristics to obtain a larger *Nicotiana rustica* giving increased yield of nicotine.

MATERIALS AND METHODS

Two inbred varieties from introduced seed lots and one of the best Olson selections were used as parents for crosses. They are described below.

(1) *Nicotiana rustica* var. *brasilia* Schrank, strain 34753 (fig. 1, *A*) Seed was obtained from A. Splendore, Scafati, Italy, in 1913, and was noted as coming originally from Brazil.

(2) *Nicotiana rustica* tall type (fig. 1, *B*). Seeds were obtained in 1926 from the Cambridge Botanical Garden, Cambridge, England.

[1] Received for publication March 3, 1941.

[2] GARRAD, G. H. THE GROWING OF TOBACCO FOR NICOTINE EXTRACTION. Southeast. Agr. Col. Jour. 20: 367-393. 1911.

They were not labeled with any varietal designation and have been called simply tall type. The strain is probably nearest to *N. rustica* var. *scabra* Cav.

(3) *Nicotiana rustica* Olson 68 (fig. 1, *C*). This line was selected from a cross between *N. rustica* var. Winnebago and *N. rustica* var. *brasilia* No. 23. It was continued by inbreeding through a number of generations until in 1938–39 the F_{10} and F_{11} were grown.

All plants in these experiments were grown at the Arlington Experiment Farm, Arlington, Va. They were placed 18 inches apart in 1-meter rows so that there were 8,800 to the acre. Shortly before planting time, 4–8–4 fertilizer was distributed uniformly in the drill

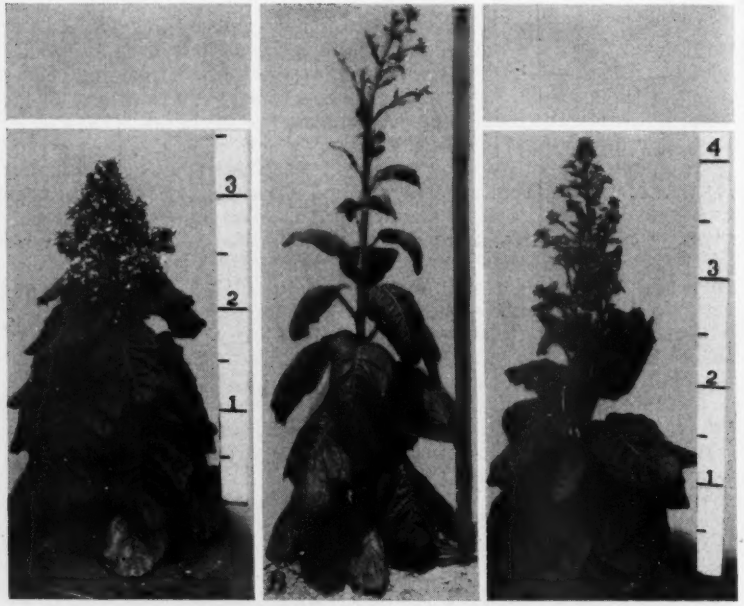

FIGURE 1.—Parental types of *Nicotiana rustica* used in crosses: *A*, Variety *brasilia*, strain 34753; *B*, tall type; *C*, Olson 68.

at the rate of 800 pounds per acre. The soil was Keyport silt loam, a heavy, claylike type, and was approximately uniform throughout the plots used.

Measurements on plant height, number of leaves, and length and width of the largest leaf were made soon after the onset of flowering. The best plants were bagged, and about 28 days later their leaves were picked and hung in the curing barn to dry.

Following the drying, or curing, selected leaf samples were weighed and ground into a fine powder preparatory to chemical analysis. A modification of the Keller method, developed in the Division of Tobacco Investigations, was used to determine the nicotine content.[3]

[3] GARNER, W. W., BACON, C. W., BOWLING, J. D., and BROWN, D. E. THE NITROGEN NUTRITION OF TOBACCO. U. S. Dept. Agr. Tech. Bul. 414, 78 pp., illus. 1934.

RESULTS ON INCREASING SIZE

PARENTS, F_1'S, AND F_2'S

Average measurements on the three parental types used are arranged in table 1. The year during which each family was grown is prefixed to its number; e. g., 37190 in 1937, 38179 in 1938. It is evident that pronounced yearly fluctuations occurred and must be taken into account when making comparisons between parents and hybrids.

TABLE 1.—*Means of measurements on parental strains of Nicotiana rustica and on F_1 and F_2 generation families resulting from crosses between them*

Parent or cross	Generation	Family No.	Number of individuals	Average plant height	Average number of leaves	Average length of largest leaf	Average width of largest leaf
				Inches		*Inches*	*Inches*
Variety *brasilia*, 34753	P_1	37190	38	28.5±0.31	19.5±0.31	13.2±0.21	10.4±0.23
	do	38179	20	29.9± .54	24.5± .82	13.1± .15	11.5± .14
	do	39232	5	14.4±1.78	11.8±2.35	9.7± .41	10.0± .65
Tall type	do	37192	68	38.4± .32	18.9± .29	12.2± .23	11.1± .29
	do	38200	24	50.0± .67	22.3± .33	12.9± .17	13.1± .29
	do	39233	5	46.0±1.30	20.2± .37	10.5± .45	10.9± .29
Olson 68	do	38186	33	32.1± .57	17.5± .52	12.9± .21	11.8± .13
	do	39238	5	30.0±2.55	14.0±1.95	10.9± .33	10.2± .41
34753 × tall type	F_1	37195	14	37.9±1.37	16.9± .27	12.6± .30	11.3± .34
	do	38203	5	47.4± .81	20.8± .58	14.3± .26	15.8± .20
(34753 × tall type) selfed	F_2	37196	70	39.2± .61	16.6± .35	13.3± .24	12.3± .19
(34753 × tall type) × 34753	do	37197	75	36.0± .86	17.7± .26	14.1± .24	12.7± .23
	do	38205	44	45.6±1.24	21.9± .16	14.6± .26	13.6± .29
(34753 × tall type) × tall type.	do	37198	71	40.5± .57	18.6± .32	12.2± .21	11.8± .22
(34753 × tall type) × Olson 68.	do	38222	80	51.9± .71	24.2± .47	14.9± .17	13.4± .16

The F_1 between strains 34753 and tall type was significantly taller than strain 34753, but was not as tall as the taller parent (table 1). It had significantly fewer leaves than either, and the largest leaf was at least as large as in the parents. Significance in this paper is determined by the t test for $P=0.05$. There was no decrease in average vigor from the first to the second hybrid generation. Backcrosses of the F_1 to strain 34753 (table 1) and to tall type tended to be intermediate between the F_1 and F_2 averages and those of the recurrent parent, with certain exceptions.

Some individuals with pronounced increases in size were obtained in the second hybrid generation. These were selfed and also were crossed with Olson 68 in an attempt to produce still larger plants. The immediate effect of Olson 68 on increasing plant height and number of leaves is evident by comparing (table 1) family 38205 with family 38222.

SELECTIONS FROM HYBRIDS

Four new lines were developed from the best plants in the second-generation families 37197 and 38222. They were continued by selfing and also were crossed once with Olson 68 and then successively selfed. Selections from the latter self-pollinations produced better plants than selections from the former. Average measurements on a few of the best families in each line, from the F_3 to the F_6, are shown in table 2. Data on some outstanding individuals are presented in table 3. The original F_2 plant of line 1 and its progeny were tall and had exceptionally large leaves, which was a major factor in producing the high yields of plant material obtained.

TABLE 2.—*Means of measurements on families in selected lines from hybrids between types of Nicotiana rustica*

Line No.	Generation	Family No.	Number of individuals	Average plant height	Average number of leaves	Average length of largest leaf	Average width of largest leaf
				Inches		*Inches*	*Inches*
Line 1	F_3	38208	69	48.2±0.98	21.2±0.45	15.5±0.19	14.2±0.20
	F_4	38224	72	51.2± .55	21.8± .25	16.3± .14	15.0± .15
	F_5	39293	5	52.2±2.58	25.2±1.59	13.1± .43	10.4± .43
Line 2	F_3	39245	8	46.3±3.03	23.8±1.54	11.9± .32	10.9± .52
	F_4	38228	42	44.6± .55	22.7± .58	15.1± .23	13.4± .20
	F_5	38278	19	50.4±1.52	27.5± .74	12.2± .22	9.6± .21
Line 3	F_4	38218	42	54.8± .83	28.0± .56	13.7± .13	12.0± .14
	F_4	38219	42	55.9± .64	27.9± .52	14.6± .18	12.4± .21
	F_5	39291	9	44.3±3.21	29.1± .83	11.3± .34	8.7± .28
Line 4	F_3	39271	10	41.7±1.27	22.9±1.07	11.7± .19	10.4± .43
	F_4	39292	6	58.7±1.48	25.0±1.16	11.7± .31	11.3± .34
Line 3 × line 1	$F_3 \times F_3$	39296	8	51.4±3.37	24.6± .87	12.9± .26	9.9± .26
Line 2 × line 1	$F_3 \times F_3$	39300	14	60.8±3.49	32.1±1.01	13.3± .26	10.7± .25

TABLE 3.—*Data on best plants in selections from hybrids*

Line and individual plant No.	Plant height	Number of leaves	Length of largest leaf	Width of largest leaf	Total weight of leaves	Nicotine, air-dry basis, plant not topped [1]	Estimated [2] potential yield of nicotine per acre if plants are topped
Line 1:	*Inches*		*Inches*	*Inches*	*Grams*	*Percent*	*Pounds*
38208–62	48	26	19.0	15.5	135.9	4.55	173
Line 2:							
39245–1	50	29	12.0	11.0	129.3	4.01	151
Line 3:							
38219–20	60	29	16.0	14.5	163.0	4.45	204
Line 4:							
38222–9	48	30	15.5	15.0	138.7	4.45	174
Line 3 × line 1:							
39296–1	36	23	12.5	9.0	112.4	5.30	159
Line 2 × line 1:							
39300–4	53	34	14.0	11.5	145.0	6.22	231
39300–6	61	41	13.5	11.0	129.0	4.90	173
39300–9	51	31	14.5	11.0	127.0	6.49	209

[1] Topping is the practice of cutting off the upper part of the plant, which includes the inflorescence and smallest leaves.

[2] Estimate based on a 2-percent increase in nicotine content if plants are topped. The yield from the stalks has not been added; this should amount to about 15 more pounds of nicotine per acre.

The original F_2 plant of line 2 and its progeny were large in all size characteristics measured and were late-flowering, a favorable feature. The original F_2 plant of line 3 and its progeny had relatively many leaves, which was a major factor in producing its high yields. Line 4 was developed from the best plant of family 38222, which was large and relatively high in nicotine content.

Many of the plants grown in 1939 were too stunted by the dry season to be considered typical; but when comparisons were made between families in which a limited number of representative individuals were measured, it was found that each new line was significantly larger than the best original types in one or more measurements. Increased size had been maintained, then, by selection and inbreeding through a number of generations and under adverse seasonal conditions.

In order to combine desirable characteristics of the new strains, crosses were made in all possible combinations among the best F_5 individuals in lines 1, 2, and 3 and the best F_5 in line 4. These F_1

crosses were each significantly larger (with one exception) than the original varieties. Especially promising was the cross between line 2 and line 1 (39300 in tables 2 and 3), which was superior to any of the new inbred lines, themselves. Line 3 × line 1 (39296) also proved to be a favorable combination.

In regard to the most outstanding segregants, plant No. 2 (fig. 2, *A*) of the cross line 2 × line 1 was 76 inches tall, which was the greatest height reached by any *Nicotiana rustica* grown during the course of

FIGURE 2.—Three exceptional plants in the family (39300) produced by crossing line 2 × line 1: *A*, Plant No. 2, height 76 inches; *B*, plant No. 6, 41 leaves; *C*, plant No. 4, 145 gm. of leaf material, estimated potential yield of 246 pounds of nicotine per acre.

the experiments. Plant No. 6 (fig. 2, *B*) of the same cross had 41 leaves, which was the highest leaf number recorded. Finally, the increased size of certain individuals was demonstrated by their high yield, which is discussed below.

RESULTS ON YIELD OF NICOTINE

WEIGHT OF LEAF MATERIAL

Selection in these experiments was based on yield of nicotine as well as on characteristics of the plant in the field. The former is mainly a function of total leaf weight and percentage of nicotine. Green plants

with the largest measurements in the field usually had the heaviest weight of dried leaf material, so that there was general correspondence between the two groups of data.

TABLE 4.—*Total weight of leaves, percentage of nicotine, and estimated potential yield of nicotine per acre from selected bagged plants of parental types of Nicotiana rustica*

Parental type	Family No.	Number of individuals	Total weight of leaves		Nicotine, air-dry basis		Estimated potential yield of nicotine per acre	
			Average	Highest	Average	Highest	Average	Highest
			Grams	*Grams*	*Percent*	*Percent*	*Pounds*	*Pounds*
Variety *brasilia*, 34753	37190	10	94.8	115.8	2.71	3.19	50.3	71.7
	38179	7	88.0	119.9	4.25	5.84	75.1	94.2
Variety *brasilia* No. 23	39231	4	41.2	63.9	6.15	7.24	47.9	68.4
Tall type	37192	9	74.5	91.2	2.09	2.73	31.5	48.3
	39233	4	44.2	50.5	3.44	4.11	28.7	34.1
Olson 68	38186	5	83.6	106.9	3.69	5.43	61.7	84.5
Olson 68A	39239	3	54.6	65.1	5.79	6.14	61.3	72.0

Weights for the parent strains are given in table 4, showing the average and maximum for each year. In 1939 data on strains 34753 and Olson 68 were not obtained, owing to an accident; so those on var. *brasilia* No. 23 and Olson 68A, two similar types, were substituted for comparison with hybrids grown that year.

TABLE 5.—*Total weight of leaves, percentage of nicotine, and estimated potential yield of nicotine per acre from F_1 and F_2 bagged plants of Nicotiana rustica hybrids*

Generation	Family No.[1]	Number of individuals	Total weight of leaves		Nicotine, air-dry basis		Estimated potential yield of nicotine per acre	
			Average	Highest	Average	Highest	Average	Highest
			Grams	*Grams*	*Percent*	*Percent*	*Pounds*	*Pounds*
F_1	37195	2	87.7	119.1	2.51	3.68	49.8	85.0
	38203	4	91.9	106.2	2.27	3.46	40.0	59.9
F_2	37196	12	98.0	120.8	3.19	4.20	61.5	86.3
	37197	24	103.2	132.2	3.11	4.22	63.2	108.2
	38205	10	95.7	125.2	3.54	4.99	66.5	104.3
	37198	16	96.8	143.6	2.85	4.56	55.6	127.0
	38222	15	101.2	143.5	3.65	5.57	72.0	119.7

[1] See table 1 for designation of parents for each family.

F_1's between strain 34753 and tall type (table 5) weighed about the same as strain 34753, the heavier parent. Best second-generation plants from the same cross were slightly heaver on an average than the F_1's and were outstanding in that the maximum weights were higher than in the parents or F_1's. This same result was obtained when germ plasm from Olson 68 was introduced (38222).

Plants in the new lines had the highest individual and average weights recorded during the experiments (tables 3 and 6). The dried leaf material alone, of the best plant in line 3, weighed 163 gm., which was the maximum weight obtained.

The best $F_5 \times F_5$ family, 39300, as judged by characteristics in the field, also had higher weights of leaf material than did any other

family grown that year. Leaves of the heaviest plant, No. 4 (fig. 2, *C*), weighed 145 gm., which compared favorably with the weight of the plants grown during better seasons.

TABLE 6.—*Total weight of leaves, percentage of nicotine, and estimated potential yield of nicotine per acre in bagged plants of best families in the new lines of Nicotiana rustica*

Line No.	Family No.	Number of individuals	Total weight of leaves		Nicotine, air-dry basis		Estimated potential yield of nicotine per acre	
			Average	Highest	Average	Highest	Average	Highest
			Grams	*Grams*	*Percent*	*Percent*	*Pounds*	*Pounds*
Line 1	38208	18	110.3	138.5	3.39	4.61	74.2	120.0
	39293	3	96.8	124.0	4.59	5.12	87.6	116.9
Line 2	39245	4	105.0	129.3	4.71	4.99	94.9	102.7
	39278	10	81.8	112.5	3.89	4.75	60.7	82.9
Line 3	38219	11	123.6	163.0	3.56	4.45	86.3	140.7
	39291	6	84.9	107.0	5.97	6.80	98.8	141.2
Line 4	39271	2	77.9	88.0	5.26	6.07	81.0	103.6
	39292	3	86.1	95.4	4.99	5.81	82.9	97.6
Line 3 × line 1	39296	4	95.3	112.4	4.10	5.30	76.9	115.6
Line 2 × line 1	39300	14	104.6	145.0	5.29	6.51	108.4	175.0

PERCENTAGE OF NICOTINE

Percentage of nicotine, like size of plant, is affected by the environment, so that pronounced yearly fluctuations occurred even in the homozygous parent strains.

Topping has a major influence on building up nicotine, and, from records over a 5-year period, it was found that topped plants had, on an average, an absolute increase of about 2.5 percent alkaloid as compared with bagged ones. An increase of 2 percent would be a conservative estimate of the effect of topping; this value corresponds closely to results obtained in New York State for 1924 and 1925.[4] Since all determinations in tables 4, 5, and 6 were made on bagged plants, approximately 2 percent should be added in order to estimate their potential content.

There was no marked gain or loss in percentage of nicotine from the higher parent to selected plants of the F_2 generation (table 5). Addition of Olson 68 in crosses caused an increase in nicotine production in both the F_2 and later generations.

The new lines were somewhat lower in percentage of nicotine than the parental types 34753 and Olson 68, but owing to their larger size some plants in most families were higher in yield of nicotine than the parents (table 6).

YIELD OF NICOTINE

Yield of nicotine per plant is the product of the weight and the percentage of nicotine. It was considered advisable to multiply this figure by the number of plants per acre, which would not change the relative individual values, and yet would provide some basis for estimating whether or not the increases were sufficiently great to be of possible economic importance.

[4] COLLISON, R. C., HARLAN, J. D., and STREETER, L. R. HIGH-NICOTINE TOBACCO. N. Y. State Agr. Expt. Sta. Bul. 562, 19 pp. 1929.

For bagged plants of the best parent strain during the years of the experiment an estimated maximum yield of about 94 pounds of nicotine per acre and an average of 75 were obtained (38179 in table 4).

The new lines contained bagged individuals with estimated yields as high as 141 pounds, and the average in a number of families was higher than in the parents (table 6). Fourteen selected bagged plants of the best $F_5 \times F_5$ family had an estimated maximum potential yield of 175 pounds of nicotine and an average of 108 pounds, obviously significant increases over the original varieties.

When the effect of topping and the yield from stalks are included, the potential values are still higher (see table 3, last column).

DISCUSSION

GENETIC CONSIDERATIONS

Increased height in intervarietal hybrids of *Nicotiana rustica* was reported by East [5] in crosses between the varieties *brasilia*, *texana*, *scabra*, and *humilis*. From the data obtained he concluded that reciprocal F_1's were very similar to each other and that the parent varieties differed by many genes for size. Hybrid vigor in first-generation plants from crosses between certain strains of *Nicotiana rustica*, but not others, was reported by Bolsunov [6] in Russia.

The F_1 described in these experiments was not larger than the parents, though other *Nicotiana rustica* crosses, less promising for nicotine production, did show typical hybrid vigor. The data reported present difficulties for genetic analysis, but certain general conclusions regarding the genetic basis for the results obtained appear tenable.

In crosses between strain 34753 and tall type it was found that the F_1 was almost as tall as the taller parent; the F_2 contained some plants that were taller than the parents or F_1; and in the F_4, families with significantly different mean heights were produced, one of which was significantly taller than tall type. These results, combined with observations on the backcrosses, were interpreted to mean that the parents differed by partially dominant genes for increasing height, most of which were from tall type. Some were contributed by strain 34753; so by genic recombination segregants and subsequent families were produced that exceeded the taller parent in height.

In crosses between strain 34753 and tall type it was found that the F_1 had fewer leaves than either parent. The F_2 generation did not differ significantly from the F_1 in average number of leaves and contained segregants that were equal, but no better, in leaf number than the original types. Backcrosses of the F_1 to each parent had significantly higher means for leaf number than the F_2. F_4 families with significantly different means occurred. Two F_4's and one F_6 had a significantly higher average number of leaves than the better parent.

It was concluded that increase in leaf number in these selections was controlled by genes, recessive in their action, some of which were contributed by each parent.

Tall type and strain 34753 do not differ significantly in length or width of the largest leaf. The F_1 was significantly larger according to 1938 results. The F_2 and first backcross generation showed a

[5] EAST, E. M. A STUDY OF PARTIAL STERILITY IN CERTAIN HYBRIDS. Genetics 6: [311]–365, illus. 1921.

[6] BOLSUNOV, I. I. CONTRIBUTION TO THE STUDY OF HYBRID VIGOUR IN NICOTIANA RUSTICA L. III DYNAMICS OF HYBRID GROWTH. Vsesoiuzn. Nauch. Issled. Inst. Tabach. i Makhor. Promysh. [Krasnodar] [Bul.] 139: [44]–57, illus. 1939. [In Russian. English summary, pp. 56–57.]

slightly increased variability over the F_1. Leaves of the backcross to strain 34753 were especially large. F_4 families were obtained that had significantly larger leaves than either parent. It was concluded that, although strain 34753 and tall type had approximately the same size largest leaf, the genes controlling this characteristic differed in the two types.

There was evidence that the genes controlling the inheritance of plant height, number of leaves, and size of the largest leaf were to some extent independent, an important factor in recombining desirable traits into new strains. The genotype of Olson 68 apparently contains more genes for increasing plant height than strain 34753; it can add to the number of leaves in certain genic combinations, and in no selections studied did the addition of Olson 68 cause a significant difference in leaf size.

In table 7, the frequency distributions of measurements on plant height, number of leaves, and length of the largest leaf are arranged for 34753, tall type, F_1, $F_1 \times 34753$, best F_4, and best $F_5 \times F_5$. The families involve only strain 34753 and tall type, with the exception of the best $F_5 \times F_5$, which contains germ plasm from Olson 68 as well as from the other two parents. Table 7 shows the variability in the families concerned and gives some idea of the effect of hybridization and of selection on the characteristics measured.

TABLE 7.—*Frequency distributions of plant height, number of leaves, and length of largest leaf for two parent types and selected hybrid families*

PLANT HEIGHT

Parent or hybrid	Frequency distribution of plant height in inches																									Mean height
	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74	76	
34753	4	2	6	8	7	3																						29.9
Tall type										1	1	4	3	5	8	1	1											50.0
F_1											1	1	3															47.4
$F_1 \times 34753$				3	1	2	1	3	3	7	3	5	2	4	0	3	4	2	0	1								45.6
Best F_4									1	1	0	2	4	4	3	3	6	9	8	1								54.8
Best $F_5 \times F_5$				1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	3	1	0	0	2	0	3	0	1	60.8

NUMBER OF LEAVES

Parent or hybrid	Frequency distribution of number of leaves															Mean number of leaves
	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	
34753	1	2	0	0	2	5	6	6	6	2						24.5
Tall type				1	6	10	7									22.3
F_1				1	2	2										20.8
$F_1 \times 34753$		1	6	2	10	8	12	4	1							21.9
Best F_4			1	1	1	0	4	10	13	5	6	1				28.0
Best $F_5 \times F_5$								1	3	3	1	5	0	0	1	32.1

LENGTH OF LARGEST LEAF

Parent or hybrid	Frequency distribution of leaf length in inches											Mean length
	10	11	12	13	14	15	16	17	18	19	20	
34753		2	5	15	8							13.1
Tall type		3	7	11	3							12.9
F_1				1	3	1						14.3
$F_1 \times 34753$	2	2	2	4	9	14	9	1	1			14.6
Best F_4	1	1	2	2	7	9	12	3	4	0	1	15.3

There were insufficient data to warrant drawing any conclusions regarding the genetic factors controlling nicotine content. It was evident, however, that either some genes for nicotine production from strain 34753 and Olson 68 were not included in the genotypes selected for the new lines, or else larger plants are not capable of building up as high a percentage of nicotine as smaller ones under the same conditions.

ECONOMIC CONSIDERATIONS

On the basis of prices paid for tobacco sold for byproduct, the returns per acre for crops containing 100 to 150 pounds of nicotine would compare favorably with the average returns over a period of years from culture of at least some types of commercial leaf tobacco. Topped plants of the original varieties actually gave such yields under the conditions described here, and the production of alkaloid may be increased somewhat by allowing the plants to remain longer in the field from time of topping to harvest. The costs of culture and of nicotine extraction are not definitely known, however, and it has been estimated that the profit, if any, from growing the original varieties for nicotine would be small.

The results on best selections in the new lines, to the F_6 generation, indicate that it may be possible to grow types of *Nicotiana rustica* that yield up to 200 pounds of nicotine per acre under the cultural and environmental conditions of these experiments. If the homozygous strains now being developed are able to produce as high yields in field tests, it should be possible to grow *N. rustica* profitably for the nicotine alone.

The cost of preparing *Nicotiana rustica* for market would be less than for commercial leaf tobacco; but, since it forms suckers more abundantly than *N. tabacum*, more labor would be required for their removal. In this connection, Bolsunov[7] has recently described an unusual variant, resulting from a cross between two types of *N. rustica*, in which the axillary buds were either partially atrophied or completely absent and in some cases of which the terminal inflorescence was also lacking. Seed was obtained from secondary suckers on the lower part of the plants, and selection to the F_5 generation has produced lines which still show the anomaly. This type, if it is as promising as described, should be of value, since the cost of topping and suckering would be reduced.

SUMMARY

New lines of *Nicotiana rustica*, developed by hybridization and selection, were significantly larger on an average than their three parental strains, namely, *N. rustica* var. *brasilia* (strain 34753), tall type, and Olson 68.

The characteristics measured were plant height, number of leaves, and size of largest leaf. These were controlled by relatively independent genes that could be recombined in advantageous groupings. The best parent values were usually not exceeded until new homozygous combinations were established in the F_2 or later generations. The original strains differed by some genes for each character.

[7] BOLSUNOV, I. I. [A VALUABLE HYBRID OF NICOTIANA RUSTICA DEVOID OF INFLORESCENCE AND UPPER SUCKERS.] Selek. i Semen. 2-3: 40-41, illus. 1939. [In Russian.]

Further increases in size were obtained by crossing F_5 selections of the new lines together. The occurrence of some exceptionally large segregants suggests that still further increases in the average size will be possible.

Related data on the weight of dried leaf material and percentage of nicotine were reported. Estimated potential yields of nicotine per acre were calculated from these figures, and selections in the new strains, to the F_6 generation, were found to be superior to their original parents in this respect. The possible economic importance of the improved lines, for use as a source of nicotine for insecticidal purposes, was discussed.

SOME FACTORS THAT INFLUENCE THE IMMEDIATE EFFECTS OF POLLEN ON BOLL CHARACTERS IN COTTON [1]

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INTRODUCTION

In 1931 Harrison (*2*)[3] reported the occurrence of immediate effects of pollen on six boll characters of cotton. As Swingle (*7*) had defined metaxenia as the immediate effect of pollen on the parts of the seed and fruit lying outside the embryo and endosperm, Harrison considered that only the characters of boll period, lint length, and seed fuzziness exhibited metaxenia. The design of the experiment performed by Harrison in 1929 was followed by the writer in experiments performed at the United States Field Station, Sacaton, Ariz., in 1934, 1935, and 1936, except that inbred strains of cotton were used exclusively. In these later experiments the effects of three kinds of pollen were compared: Pima Egyptian from a strain inbred 20 years, Acala upland from a strain inbred 11 years, and Hopi from a strain inbred 12 years, these pollens having been used to fertilize emasculated flowers of Acala of the same strain as that which furnished the Acala pollen.

Differences of smaller magnitude than those reported by Harrison, but usually significant, were found in respect to each of the five characters determined in these later experiments. Kearney (*3*) in 1929 and Harrison (*2*) in 1931 reported that boll-maturation period was affected by the amount of exposure to sunlight. The existence of other factors that can mask or emphasize the expression of the immediate effects of pollen on cotton was demonstrated by the experiments reported in this paper. The factors referred to are the effects of (1) variation in seasonal conditions from year to year, (2) variations in weather or other conditions on or near the day of anthesis, (3) individual variation among the plants used as pistillate parents, and (4) variation in conditions affecting the nutrition of the bolls.

MATERIALS AND METHODS

It was sought to determine the effects of pollen in bolls resulting from two interspecific cross-pollinations, A × P (*Gossypium hirsutum L.* × *G. barbadense L.*, Acala × Pima) and A × H (*G. hirsutum* × *G. hopi* Lewton, Acala × Hopi), and one endogamic cross-pollination, A × A (*G. hirsutum* × *G. hirsutum*, Acala × Acala) in respect to five characters of the bolls, namely, number of seeds, seed index,[4] lint index,[5] fiber length, and boll-maturation period.

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[2] The author is indebted to E. Gordon Smith, assistant scientific aide, Division of Cotton and Other Fiber Crops and Diseases, for generous assistance in the field and in the laboratory.

[3] Italic numbers in parentheses refer to Literature Cited, p. 480.

[4] Seed index is the equivalent of the weight, in grams, of 100 seeds.

[5] Lint index is the equivalent of the weight, in grams, of the lint removed from 100 seeds.

Fifteen Acala plants were selected as pistillate plants each year, and each plant received the 3 cross-pollinations in rotation as flowers appeared throughout the pollinating season. Fifty undamaged bolls of each cross-pollination were obtained in 1934 and in 1936, but the 1935 material was discarded, except for fiber-length determinations from undamaged locks, because of the very small number of normal bolls obtained before frost. In 1934 the 150 bolls were obtained from pollinations made on the 10 days (Saturday and Sunday excepted) between August 15 and August 27. In 1936 they were obtained from pollinations made on 18 days between August 1 and August 27.

INTERRELATIONSHIPS OF BOLL CHARACTERS STUDIED

Table 1 gives the set of coefficients of correlation for the 5 boll characters considered in this study. The "within seasons" value of r is used in order to have a figure that would most nearly correspond to one that could be expected if all 300 bolls had been grown in one year. Number of seeds per boll is negatively correlated with seed index, with lint index, and with fiber length. The fact that fewer seeds per boll resulted in heavier seeds with more abundant and longer lint indicates that a nutritional factor is involved. Assuming that a definite quantity of plant food is furnished each boll, it is a logical assumption that a few seeds utilizing this food would grow larger and produce more and longer lint than if the same amount of food were utilized by a larger number of seeds.

TABLE 1.—*"Within seasons" value of the correlation coefficients of characters determined on 300 bolls of cotton grown on Acala plants in 1934 and in 1936 as a result of cross-pollinations A × P, A × A, and A × H*

Character and population [1]	Correlation with—			
	Seed index	Lint index	Fiber length	Boll period
Seeds per boll:				
As one array	−0.6899	−0.3496	−0.3223	−0.0001
A × P	−.6499	−.2424	−.3055	+.0680
A × A	−.8290	−.4345	−.3554	+.1175
A × H	−.6764	−.2646	−.3377	−.0563
Seed index:				
As one array		+.3741	+.5491	−.1548
A × P		+.5356	+.7117	−.2771
A × A		−.0088	+.3608	−.2200
A × H		+.3445	+.5210	−.0820
Lint index:				
As one array			+.1834	+.1067
A × P			+.4243	+.0374
A × A			+.0704	+.1173
A × H			−.0257	+.1195
Fiber length:				
As one array				−.1745
A × P				−.2397
A × A				−.1348
A × H				−.1670

[1] In "As one array," where $N=300$, r should be ± 0.148 at $P=0.01$; in the individual cross-pollinations, where $N=100$, r should be ± 0.254 at $P=0.01$ (8), N=number of observations.

The correlation coefficients of seed index with lint index show that, with the exception of cross-pollination A × A, heavy seeds are associated with abundant fiber. All three cross-pollinations produced longer fiber on heavier seeds than on light seeds. The negative correlation of seed index with boll period in cross-pollination A × P and A × A shows that as a rule heavier seeds mature faster than

lighter ones. The lack of correlation of seed index with lint index in cross-pollination A × A and the existence of a correlation of seed index with boll period when there was no correlation of number of seeds per boll with boll period indicate that heterosis is also involved. The heavy seeds in cross-pollinations A × P and A × H are apparently larger and have a more extensive surface from which to develop fibers. The lack of correlation of seed index with boll period in the material from cross-pollination A × H, although such a correlation resulted in cross-pollinations A × P and A × A, demonstrates the difficulty of segregating the effects of heterosis from those of nutrition.

As Pope (*4, pp. 744–747*) has shown a relatively uniform weight per unit length of fiber in the Acala group, the increased weight of fibers on the heavy seeds might be due in part to the increased length. However, only cross-pollination A × P showed a significant positive correlation of lint index with fiber length.

In the three populations as one array, the negative correlation of fiber length with boll period shows that the factors that promote the development of long fibers tend to hasten maturity, adding another indication that nutrition is a factor involved in these results.

The use of inbred strains of cotton to eliminate significant variation among plant means was only partly successful. Table 2 shows that for each of the five characters the differences between means of the pistillate plants receiving all cross-pollinations ranged from zero to a maximum greater than the maximum difference between the means of the populations receiving the several cross-pollinations. A record of the staminate parents was made in 1936. There was no significant difference in number of seeds per boll among means of staminate plants furnishing pollen for any of the three cross-pollinations. In view of the highly significant correlation of this character with seed index, with lint index, and with fiber length, it was assumed that a uniform supply of pollen had been used for each cross-pollination. The small number of staminate plants involved, five of Pima, four of Acala, and two of Hopi, undoubtedly reduced the opportunity for wide variation among their means.

TABLE 2.—*Range of differences and average of differences among means of cross-pollinations, between means of seasons, among means of day of anthesis within seasons, and among means of pistillate plants within seasons for each of the 5 characters determined on 300 bolls of cotton produced by Acala plants in 1934 and in 1936 as a result of pollination with Pima, Acala, and Hopi pollens*

Means compared	Number of comparisons	Character									
		Seeds per boll		Seed index		Lint index		Upper quartile fiber length		Boll period	
		Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Cross-pollination	3	2.53–5.77	3.85	0.11–1.06	0.71	0.05–0.34	0.23	0.003–0.016	0.011	0.23–0.95	0.63
Seasons	1		2.16		.77		.24		.046		3.23
Day of anthesis:											
1934	45	0 –11.5	4.69	.01–2.60	1.06	0 –1.11	.37	0 – .130	.052	.10–6.70	2.65
1936	153	0 – 7.7	2.21	.01–2.14	.60	0 –1.39	.45	0 – .080	.023	0 –16.0	6.36
Pistillate plants:											
1934	105	1 – 7.1	2.55	0 –2.2	.72	0 –1.2	.32	0 – .056	.021	0 – 5.9	1.70
1936	105	0 –10.8	2.47	0 –1.1	.43	0 – .9	.30	0 – .038	.013	0 –12.5	3.43

EFFECTS OF CROSS-POLLINATIONS

In the comparison of means of cross-pollinations shown in table 3, the differences between cross-pollinations A × P and A × A are significant in respect to all five characters. The use of Pima pollen on Acala plants, in comparison with the use of Acala pollen on the same plants, reduced the number of seeds per boll, increased the weight of the seeds, increased the amount of lint on the seeds, lengthened the fibers, and required a longer time to mature the bolls.

Hopi pollen, as shown in the comparison of cross-pollinations A × H and A × A, increased significantly the number of seeds per boll and the fiber length. The differences between means of seed index, lint index, and boll period were not significant. The increased fiber length in cross-pollination A × H was the reverse of the results obtained by Harrison (*2*). As he used the same strain of Hopi as that used in the experiments reported in this paper, the consistent increase in length obtained in 1934, 1935, and 1936 was unexpected. This apparent reversal of the effects of Hopi pollen on fiber length will be discussed further in the section on effects of specific pistillate plants.

When cross-pollinations A × P and A × H are compared, significant differences between means of all characters except fiber length are shown. Cross-pollination A × P yielded fewer seeds per boll than cross-pollination A × H and gave larger means for seed index, lint index, and boll period.

TABLE 3.—*Comparison of the means* [1] *of cross-pollinations for each of the five characters determined on 100 bolls of cotton of each of the three cross-pollinations, A × P, A × A, and A × H, grown in 1934 and 1936*

Cross-pollination	Character									
	Seeds per boll (number)		Seed index (grams)		Lint index (grams)		Upper quartile fiber length (inches)		Boll period (days)	
	M	*t*	*M*	*t*	*M*	*t*	*M*	*t*	*M*	
A × P	25.57		12.79		6.70		1.0288		58.25	
A × A	28.81		11.84		6.36		1.0129		57.53	
Difference	−3.24±0.373	8.69	+.95±0.063	15.08	+.34±0.045	7.56	+0.159± 0.0034	4.68	+.72±0.267	2.70
A × H	31.34		11.73		6.41		1.0262		57.30	
A × A	28.81		11.84		6.36		1.0129		57.53	
Difference	+2.53± .367	6.89	−.11± .062	1.77	+.05± .044	1.14	+.0133± .0033	4.03	−.23± .262	.88
A × P	25.57		12.79		6.70		1.0288		58.25	
A × H	31.34		11.73		6.41		1.0262		57.30	
Difference	−5.77± .365	15.81	+1.06± .062	17.10	+.29± .044	6.59	+.0026± .0033	.79	+.95± .261	3.64

[1] See Wallace and Snedecor (*8, pp. 63-65*) for a table of *t* values.

FACTORS THAT MODIFY THE IMMEDIATE EFFECTS OF POLLEN

VARIATIONS IN SEASONAL CONDITIONS FROM YEAR TO YEAR

Table 2 shows that the difference between the means of seasons in number of seeds per boll is smaller than the minimum difference between means of cross-pollinations. In seed index and in lint index the difference between means of seasons approximates the average of the three differences among means of cross-pollinations. In fiber length and in boll period the difference between means of seasons is approximately three times the maximum difference between means of cross-pollinations.

The influence of season in modifying or changing the effects of different pollens is best illustrated in seed index, where cross-pollination and season interaction exists (see table 4). The difference of 0.77 gm. between means of seasons shown in table 2 represents an increase in 1936 equal to 6.7 percent of the 1934 mean of all cross-pollinations as one array. The increase in cross-pollination A × P was 3.8 percent of its 1934 mean, that in cross-pollination A × A was 10.4 percent of its 1934 mean, and that in cross-pollination A × H was 6.2 percent of its 1934 mean. By extending these experiments over two seasons, the magnitude of the differences among means of cross-pollinations in seed index is smaller than would have been the case if only the results of 1934 had been used.

TABLE 4.—*Level of significance of differences among means of factors affecting boll characters of cotton as shown by analyses of variance of 100 bolls each of 3 cross-pollinations grown in 1934 and in 1936 (group A), and of 15 bolls of each of 3 cross-pollinations, 3 bolls of each cross-pollination with 29, with 30, with 31, with 32, and with 35 seeds per boll, grown in 1936 (group B)*

Items	Differences among means of factors affecting characters indicated				
	Number of seeds	Seed index	Lint index	Upper quartile fiber length	Boll period
Group A:					
Cross-pollinations	<0.01	<0.01	<0.01	>0.05	0.05
Seasons	<.01	<.01	<.01	>.05	<.01
Day of anthesis	<.01	<.01	<.01	<.01	<.01
Pistillate plants	.05	0.05-.01	0.05-.01	>.05	>.05
Bolls with many and few seeds	<.01	<.01	.01	<.01	>.05
Interactions:					
Cross-pollinations—seasons	>.05	<.01	>.05	>.05	>.05
Cross-pollinations—day of anthesis	<.01	<.01	>.05	>.05	>.05
Cross-pollinations—pistillate plants	>.05	<.01	>.05	.05-.01	.05-.01
Cross-pollinations—many and few seeds	<.01	.05	.05	>.05	>.05
Seasons—many and few seeds	.01	<.01	>.05	<.01	>.05
Cross-pollinations—seasons—many and few seeds	<.01	<.01	>.05	----------	>.05
Group B:					
Cross-pollinations	----------	>.05	>.05	>.05	>.05
Seeds per boll	----------	>.05	>.05	>.05	>.05
Interaction:					
Cross-pollinations—seeds per boll	----------	>.05	>.05	>.05	>.05

VARIATIONS IN WEATHER OR OTHER CONDITIONS ON OR NEAR THE DAY OF ANTHESIS

The average and maximum differences among means of day of anthesis, shown in table 2, seem large when the short periods of time during which pollinations were made are considered. In all five

characters the maximum difference between means of day of anthesis in each season was larger than the maximum difference between means of cross-pollinations. Some weather phenomena of the day of anthesis, or of the days preceding or following it, are presumed to have contributed to these effects; or there may have been a cumulative effect of some phenomena operating over a period of several days preceding, or possibly following, the day of anthesis. Unsuccessful attempts were made to correlate the number of seeds per boll with maximum, with minimum, and with average temperatures, and with the average relative humidity percentage of the day of anthesis. There was a slight indication that number of seeds per boll and evaporation from an open tank 5 days after anthesis might be negatively correlated, but the value of r was far from significant. A study of the average yields of American-Egyptian cotton in Arizona showed a negative correlation of yields with July and August evaporation from an open tank at Sacaton (*1*).

The importance of the effects of day of anthesis on number of seeds per boll in this study can be illustrated by examples of cross-pollination and day of anthesis interaction, or the differential response of the three pollens to the conditions of the day, or of days near the day, on which the crosses were made. On days 4 and 5 in 1934 (August 18 and 21) the mean numbers of seeds per boll were 23.0 and 29.6 respectively, a difference of 6.6 seeds. This difference of 6.6 seeds per boll was due to the fact that cross-pollination A × P yielded on the average 13.2 more seeds per boll on day 5 than on day 4; cross-pollination A × A responded with an average of 11.6 more seeds per boll; but cross-pollination A × H gave an increase of only 1.6 seeds per boll. Thus, although the conditions on day 5, in comparison with day 4, were advantageous to all three kinds of pollen, cross-pollinations A × P and A × A responded to a much greater extent than cross-pollination A × H. If conditions similar to those of day 5 had existed throughout the entire pollination period, the means for the several cross-pollinations at the end of the season would have been greatly changed. In 1936 the mean numbers of seeds per boll on days 14 and 15 (August 22 and 23) were 32.2 and 28.2 respectively, an average difference of 4.0 seeds per boll. Cross-pollination A × P gave an average of 5.4 fewer seeds on day 15 than on day 14, and cross-pollination A × A showed an average decrease of 7.7 seeds per boll; but cross-pollination A × H averaged 1.0 seed more per boll on day 15 than on day 14. If the conditions that existed on day 15 in 1936 had predominated throughout the entire pollination period, cross-pollinations A × H would have had a decided advantage over cross-pollinations A × P and A × A in number of seeds per boll.

The effect of day of anthesis on seed index showed day 5 in 1934 with a decrease in seed index from day 4 of 0.43 gm. for cross-pollination A × A, of 1.57 gm. for cross-pollination A × H, and of 3.58 gm. for cross-pollination A × P. In 1936 day 15 showed an increase in seed index over day 14, cross-pollination A × H increasing only 0.10 gm., and cross-pollination A × P only 0.15 gm., but the mean seed index of cross-pollination A × A was increased by 1.37 gm. Cross-pollination and day of anthesis interaction is not significant in the three remaining characters.

VARIATION AMONG PLANTS USED AS PISTILLATE PARENTS

Differences among the means of pistillate plants were above the 5 percent level of significance in fiber length and in boll period, and on the 5-percent or between the 5-percent and 1-percent level in number of seeds per boll, in seed index, and in lint index (table 4). However, the differences in fiber length among the means of pistillate plants provides an explanation of the unexpected lengthening effects of cross-pollination A × H in this character and will be used to illustrate these effects.

Two plants of each season were selected at random for this purpose. These are plants 5 and 9 of 1934 and plants 7 and 8 of 1936. In 1934 the average upper quartile fiber lengths of plants 5 and 9 were 0.98 and 1.00 inch respectively, plant 9 having bolls with fiber 0.02 inch longer than plant 5. Cross-pollinations A × A and A × H both gave fibers averaging 0.01 inch longer on plant 9 than on plant 5, while fibers of cross-pollination A × P were 0.06 inch longer on plant 9. In 1936 there was no difference between the means of plants 7 and 8 for bolls of cross-pollinations A × A and A × H, the small difference of cross-pollination A × P being only 0.002 inch in favor of plant 7.

An examination of plant means of the 1934 material showed that 1 plant did not produce bolls of both cross-pollinations A × A and A × H, and of the remaining 14 plants, 5 had means of cross-pollination A × H smaller than those of cross-pollination A × A. In 1936, 2 of the pistillate plants did not have bolls of both of these cross-pollinations, 1 plant had the same mean for both, 6 plants had means of cross-pollination A × A exceeding those of cross-pollination A × H, and there were 6 plants in which cross-pollination A × H exceeded cross-pollination A × A in fiber length. The differences on the 6 plants where the fibers of cross-pollination A × H were longer than those of cross-pollination A × A were large enough to mask entirely the effects shown on the other 6 plants where the differences were reversed.

Designating Harrison's (*2*) cross-pollination Durango × Durango as D × D, and his cross-pollination Durango × Hopi as D × H, where he used 25 Durango plants as pistillates in 1929, an examination of his original records shows that 13 of the 25 plants gave means for fiber length that were higher in D × H than in D × D. These include 50 of the D × H bolls, with a mean of 4.90 grades, and 52 of the D × D bolls, with a mean of 4.78 grades. The difference of 0.12 grade is equivalent to a lengthening effect of Hopi pollen of 0.015 inch. On the other 12 pistillate plants the 50 D × H bolls had a mean fiber length of 4.36 grades and the 48 D × D bolls a mean of 4.84 grades. The difference of 0.48 grade, or 0.06 inch, was sufficiently large to mask the lengthening effect of the Hopi pollen exhibited by the other 13 plants.

Pressley (*6, pp. 272–273*) also demonstrated an influence of individual differences in pistillate plants upon the effects of different pollens, his unit of comparison being a pair of seeds from near the

center of a lock, one selfed the other a hybrid.[6] Thus his selfed and hybrid seeds had a maximum of uniform conditions for growth and development. In his comparison of Pima selfed with Pima-Red Acala hybrids, the mean fiber length of the selfed seeds on plant 7 was 9/128 inch longer than those on plant 2, while the hybrid seeds of these pairs averaged 16/128 inch longer on plant 7 than on plant 2.

These examples of the effects of differences in individual pistillate plants as to fiber length show that effects of the pollen used may be modified or completely reversed by the selection of the pistillate plants used in the experiment.

VARIATION IN CONDITIONS AFFECTING NUTRITION

The correlations existing in the material used in these experiments, especially those of number of seeds per boll with seed index, with lint index, and with fiber length, indicate that nutrition was a factor affecting the expression of these characters. In 1929 Kearney (*3*) reported that the removal of the involucre of Pima cotton at the time of anthesis caused a marked reduction in the size and weight of the boll, in the weight of seeds, and in the abundance of lint. When the involucre was removed about 12 days before anthesis, the same was true and there was also a reduction in the number of seeds in the boll, presumably due to the undernutrition and resulting sterility of some of the ovules. In order to obtain the significant differences that Kearney found, it must be assumed that the Pima cotton plant furnishes food to the individual boll within a limited range; otherwise the effect of removing the involucre from the bolls would have been masked by the bolls receiving additional food from the unlimited central supply of the plant.

In table 5 the 1934 and 1936 material has been regrouped into classes of bolls with "many seeds" and with "few seeds." Table 6 gives the range of the number of seeds in each of these two classes. When the material from the three cross-pollinations is taken as one array, highly significant differences are shown between the means of bolls with relatively few and relatively many seeds in the characters, seed index, lint index, and fiber length. In cross-pollinations A × P and A × A significant differences between means for bolls with few and with many seeds are shown only in seed index and in fiber length. In cross-pollination A × H the only significant difference was in seed index. These data clearly show that a reduction in the number of seeds per boll results in heavier seeds that have longer and possibly more abundant lint. Porter (*5*), working with 10 varieties or strains of upland cotton grown at Greenville, Tex., in the years 1931, 1932, and 1933, found that number of seeds per lock was negatively correlated with fiber length. This is another illustration of a reduction in number of seeds resulting in longer lint.

[6] Pressley applied pollen of 2 varieties of cotton to the stigmas of his pistillate plants. From the resulting bolls he worked up data on a large number of pairs of seeds, each pair from near the center of an individual lock. The next season these seeds were grown to determine their male parentage, and only data from those pairs that consisted of 1 self-fertilized seed and 1 cross-pollinated seed were used in reporting his experiment.

TABLE 5.—*Comparison of the means*[1] *of bolls with "few" seeds and of bolls with "many" seeds for each of the 5 characters determined on the 100 bolls of each cross-pollination grown on Acala plants in 1934 and 1936*

Means compared	Bolls	Seeds per boll	Seed index	Lint index	Upper quartile fiber length	Boll period
All cross-pollinations combined:	*Number*	*Number*	*Grams*	*Grams*	*Inches*	*Days*
Bolls with few seeds	154	24.5	12.66	6.59	1.035	57.4
Bolls with many seeds	146	32.8	11.54	6.39	1.009	58.0
Difference		−8.3±.41	1.12±.125	.20±.071	.026±.0057	−.6±0.60
t		20.24	8.96	2.82	4.56	1.00
Cross-pollination A × P:						
Bolls with few seeds	54	21.1	13.46	6.79	1.041	57.5
Bolls with many seeds	46	30.8	11.96	6.59	1.013	59.1
Difference		−9.7±.72	1.50±.217	.20±.123	.028±.0098	−1.6±1.04
t		13.47	6.91	1.63	2.86	1.54
Cross-pollination A × A:						
Bolls with few seeds	47	24.4	12.39	6.42	1.028	57.0
Bolls with many seeds	53	32.7	11.35	6.31	1.000	58.1
Difference		−8.3±.72	1.04±.217	.11±.123	.028±.0098	−1.1±1.04
t		11.53	4.79	.89	2.86	1.06
Cross-pollination A × H:						
Bolls with few seeds	53	28.1	12.08	6.52	1.035	57.7
Bolls with many seeds	47	34.9	11.34	6.28	1.015	56.9
Difference		−6.8±.72	.74±.217	.24±.123	.020±.0098	.8±1.04
t		9.44	3.41	1.95	20.4	.77

[1] See Wallace and Snedecor (*8, pp. 62–63*) for a table of *t* values.

TABLE 6.—*Range in number of seeds per boll in "many-few" classification given in table 5*

Cross-pollination	Bolls produced in 1934 with—		Bolls produced in 1936 with—	
	Few seeds	Many seeds	Few seeds	Many seeds
A × P	9–23	24–36	13–28	29–42
A × A	14–28	29–40	16–30	31–36
A × H	4–31	32–42	16–32	33–38

If the immediate effect of different pollens were determined by nutrition alone, and if each cross-pollination were represented by an equal number of bolls, all having the same number of seeds per boll, there would be no differences among means of cross-pollinations. In the 1934 material the frequency distribution of number of seeds per boll was so irregular that 2 was the maximum number of bolls that would equally represent all three cross-pollinations, and that was available in only the class of 29 seeds per boll. In the 1936 material it was possible to select 3 bolls of each cross-pollination in each of 5 classes, those of 29, 30, 31, 32, and 35 seeds per boll. The means of these 15 bolls from each cross-pollination are compared in table 7. There are no significant differences among means of cross-pollinations in any of the 4 characters, although the difference between the mean lint index of cross-pollinations A × H and A × A approaches significance. Although the populations are small in the comparisons presented in table 7, the effects of seasons and of

number of seeds per boll have been eliminated by selection of material. If it were possible to eliminate also the effects of day of anthesis and of variations among individual pistillate plants, the importance of nutrition in modifying effects of different pollens might be more clearly seen.

TABLE 7.—*Comparison of means*[1] *of 15 bolls of each cross-pollination, 3 bolls of each with 29, with 30, with 31, with 32, and with 35 seeds per boll, for the 4 remaining characters determined on bolls grown in 1936*

Cross-pollination	Seed index	Lint index	Upper quartile fiber length	Boll period
	Grams	*Grams*	*Inches*	*Days*
A × P	12.51	6.71	1.039	59.5
A × A	12.29	6.30	1.033	59.5
Difference	.22±.267	.41±.228	.006±.0133	.0±2.25
t	.82	1.80	.45	
A × H	12.23	6.77	1.040	58.6
A × A	12.29	6.30	1.033	59.5
Difference	−.06±.267	.47±.228	.007±.0133	−.9±2.25
t	.22	2.06	.53	.40
A × P	12.51	6.71	1.039	59.5
A × H	12.23	6.77	1.040	58.6
Difference	.28±.267	−.06±.228	−.001± 0133	.9±2.25
t	1.05	.26	.01	.40

[1] See Wallace and Snedecor (*8, pp 62-63*) for a table of *t* values.

The differences between the means of cross-pollinations A × P and A × A, and a study of the correlations existing among the characters in each of these cross-pollinations, indicate that nutrition, as determined by a differential in ability of the two pollens to effect fertilization of the ovules, is a primary factor in determining the immediate effect of pollen. There is a possibility that physical crowding, instead of nutrition, may be the primary effect of number of seeds per boll. However, the correlations existing in cross-pollination A × H and a comparison of the differences between the means of A × H and A × A with the differences between the means of A × P and A × A indicate that nutrition exerts a greater influence than physical crowding. When the means of cross-pollination A × H are compared with those of cross-pollinations A × A or A × P, the presence of factors other than nutrition becomes evident. The effects of seasons, of day of anthesis, of individual pistillate plants, and of nutrition are all intermingled in their modification of immediate effects of pollen, so that it is impossible to rank them in the order of their importance.

SUMMARY

Strains of cotton inbred from 11 to 20 years or more were used to test the immediate effects of pollen on boll characters. Emasculated flowers of Acala cotton were pollinated with (1) Pima pollen, (2) Acala pollen, and (3) Hopi pollen, the Acala pollen having been taken from plants of the same inbred strain that comprised the pistillate plants receiving all three cross-pollinations. Significant differences among means for the several cross-pollinations were obtained

in number of seeds per boll, in seed index, in lint index, in fiber length, and in boll-maturation period.

It is shown that effects upon the expression of these characters by differences between (1) different years, (2) different days of anthesis in the same year, and (3) different individual plants used as pistillate parents are so great as conceivably to mask completely the effects, if any, of different pollens. The difference between means for different years approximated the average difference between means for the several cross-pollinations in all characters except number of seeds per boll. In this character the difference, although highly significant, was smaller than the minimum difference between means of cross-pollinations. In both seasons the maximum difference among means for successive days of anthesis and among means for each of the individual pistillate plants exceeded the maximum difference among means of the several cross-pollinations.

An assumed nutritional factor was shown to affect the expression of seed index, of fiber length, and probably of lint index. This suggests that a difference among the several pollens in ability to fertilize a high percentage of the ovules was another factor that determines the effects of cross-pollinations.

The influence of these various factors, and undoubtedly of others not covered by this study, are so intermingled that it is impossible to rank them in the order of their importance.

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CHLORATE TOXICITY AND PERSISTENCE IN RELATION TO SOIL REACTION [1]

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INTRODUCTION

There is evidence from various sources of lesser toxicity of chlorate in alkaline solutions. Thus Offord and d'Urbal (*17*)[2] reported that sodium chlorate was more toxic to *Nitella* in acid solutions than in neutral or alkaline ones. They used as their criterion of toxicity the time required for the outward diffusion of 50 percent of the vacuolar chloride, and found it to be correlated with the pH value of the solution. At pH 4.8, the time required was 38 hours; at pH 5.2, 56 hours; at pH 5.8, 81 hours; at pH 6.6, 87 hours; at pH 7.4, 120 hours; and at pH 8.2, 125 hours. In agreement with this relation, Yamasaki (*20*) found that potassium chlorate was most injurious to wheat and rice seedlings in solutions made acid and least injurious in those made alkaline. Hulbert, Bristol, and Benjamin (*10*) discovered that sodium chlorate wilted cut plants most rapidly in acid solutions (pH 6.0 or below), but they were unable to show with certainty that the toxicity of chlorate sprays was enhanced by acidification. However, other field tests (*4*, *7*, *16*) have proved that chlorate sprays are most effective as herbicides when made slightly acid. And finally Grau (*9*) found that the application of lime to soil retarded the killing action of chlorate and apparently somewhat increased the length of time the chlorate was effective.

The purpose of the present investigations was to determine the importance of soil reaction, first as a factor in the effectiveness of sodium chlorate added to the soil for weed control, and secondly as a factor in the persistence of the chlorate in treated soils.

MATERIAL AND METHODS

Water-culture experiments were carried out with seedlings of soybean (Palmetto), sunflower, and barley (Hannchen) grown in acid, neutral, and alkaline solutions containing magnesium sulfate, calcium nitrate, ferric citrate, and mono-, di-, and tribasic potassium phosphate in such proportions as to buffer the solutions at the desired reactions. Acidity was checked colorimetrically every day or every other day, and in the first experiments was maintained by frequent change of solutions. Later the reaction of the acid cultures was adjusted as necessary by adding a little phosphoric acid, and that of the alkaline ones by adding sodium hydroxide or a small amount

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[2] Italic numbers in parentheses refer to Literature Cited, p. 493.

of saturated sodium bicarbonate solution. Nitrogen concentrations were relatively low, 37 p. p. m., thus enhancing the toxicity of the chlorate (*8, 13*).

The seedlings were germinated in quartz sand and transferred to the nutrient solutions when about 6 cm. high. Relative degrees of injury were estimated from leaf symptoms and from degrees of stunting.

Soil experiments with corn (*Zea mays*), sorgo (*Sorghum vulgare*), millet (*Setaria italica*), soybean (*Soja max*), sunflower (*Helianthus annuus*), and barley (*Hordeum vulgare*) were carried out with a composted Keyport clay loam. The greenhouse benches were divided by means of board partitions into plots about 3 feet square (surface area 10 square feet) each of which contained about 400 pounds of soil (325 pounds on an oven-dry basis). Some of the plots were made alkaline (pH 7.5–8.0) by the addition of slaked lime at a rate of 1, or in some experiments, 2 pounds, per plot. Others were acidified to near pH 5.2 with about 400 cc. of sulfuric acid, much diluted and sprinkled on the soil with a watering can. Others were left at the natural reaction, always approximately neutral. After the lime or sulfuric acid was thoroughly mixed with the entire mass of soil, sodium chlorate in solution was similarly well mixed into half of the plots of each reaction, the rest being left as controls. Fifteen grams of chlorate were added to each plot except in the case of the experiment with sunflower in table 3, where the quantity was half as much. By measuring the water applications throughout the experiments, soil moisture was kept fairly uniform and leaching avoided.

The acidity of the soil was determined at the beginning and end of each experiment. Approximately 100 gm. of soil were shaken with 100 cc. of water in a wide-mouthed bottle and allowed to settle overnight, or until the supernatant extract was clear. When the soil was unusually wet, the amount of water was reduced to 90, or in extreme cases, to 80 cc., so that the ratio of water to weight of soil was always approximately 1 to 1. Five-cubic-centimeter samples were carefully pipetted from the clear water layer for colorimetric determination of pH by means of the Taylor slide comparator.

To obtain plants as uniform in size as possible, extra seeds were sown and the smaller seedlings discarded soon after germination. The same number of plants of a given crop was always left in each row of the plots to be compared.

In order to correct for differences in size that might be caused by differences in the soil reaction, degrees of chlorate injury are indicated in the tables by the magnitude of the so-called growth percentage, i. e., the average green weight of the tops of an entire culture or plot expressed as a percentage of the corresponding weight for control plants in a corresponding culture or plot without chlorate. Relative amounts of injury were also apparent from the degree of leaf discoloration, for chlorosis and, with severe injury, necrosis were most severe on the smaller, more stunted plants. Wherever growth percentages were near 100, indicating normal size, leaf color was also normal. Absolute weights of the control plants are given in the columns to the right in each table in order to show the effect of the soil reaction itself. The recording of these weights also makes possible calculation of the absolute weights of the chlorate-injured plants.

RESULTS

CHLORATE TOXICITY IN RELATION TO ACIDITY

Symptoms of injury by chlorate were distinct and easily recognized on all the crops. Chlorosis, with brown spots that often became necrotic, was characteristic, and the lower leaves died. On barley there was a grayish withering of the leaf tips, extending eventually, in severe injury, to the entire leaf. In water cultures these symptoms appeared most quickly if the plants were allowed to develop a good root system before being transferred to solutions containing chlorate; so the procedure was adopted of allowing them to grow for a week, after germination in sand, in a solution having a favorable, approximately neutral reaction. Control plants grown without chlorate always prevented confusion of symptoms due to chlorate with those due to an unfavorable reaction of the solution.

The controls showed that near-neutral and alkaline solutions were most favorable for growth of soybean and sunflower, respectively, acidity as high as pH 5.0 being highly injurious. Barley grew quite uniformly in all the solutions, tolerating acidities as high as pH 5.0 much better than either soybean or sunflower. Some chlorosis from iron deficiency occurred occasionally in alkaline solutions, but it was not serious in the 2-week period of the experiments.

Several preliminary experiments with wheat showed no effect of the solution reaction on chlorate toxicity. But subsequent trials with soybeans grown under similar conditions showed definitely that the leaf symptoms produced by 25 p. p. m. of sodium chlorate were most severe where the nutrient solution was below pH 6.0, less severe where it was between pH 6.0 and 7.0, and slight or absent between pH 7.2 and 8.3. Sunflower also proved to be most severely injured by chlorate in the acid solution. Because of the poor growth of the control plants in the more acid solutions, subsequent tests were carried out with barley, which grew well over a wider range of reactions.

For the first of the experiments with barley, seedlings grown for a week in a near-neutral solution to develop uniform root systems were transferred to solutions containing 0, 10, 20, 40, and 50 p. p. m. of chlorate, respectively. Each series was replicated at acid (pH 5.1–6.2), neutral (pH 6.8–6.9), and alkaline (pH 9.1–8.3) reactions. After 3 days there were distinct symptoms of chlorate injury in the acid and near-neutral solutions at all chlorate concentrations except the lowest, 10 p. p. m. In the alkaline solution no symptoms were detectable at this time at any chlorate concentration except the highest, 50 p. p. m. Two days later, injury had appeared here with 40 and 30 p. p. m. also, but to a much lesser degree than at these concentrations in the neutral and acid solutions. The contrasting appearance of the acid and alkaline series after 7 days is illustrated in figure 1. The control plants for the two reactions (extreme left in each series) look alike.

After 11 days the plants were cut, and those grown with 10 p. p. m. of chlorate at the acid, neutral, and alkaline reactions were photographed. Figure 2, *A*, shows that the chlorate injured the plants in the acid solution severely, those in the near-neutral one moderately, while those in the alkaline solution were apparently uninjured.

In another experiment the lower leaves of barley plants in the acid solution (pH 5.0–5.4) began to wither 2 days after the addition of 40

p. p. m. of chlorate to the cultures, whereas those in the neutral and alkaline solutions evinced no ill effects. Two days later, when the photograph shown in figure 2, *B*, was made, the lower leaves of the plants in the neutral solution (pH 7.0–6.6) were also withered, but

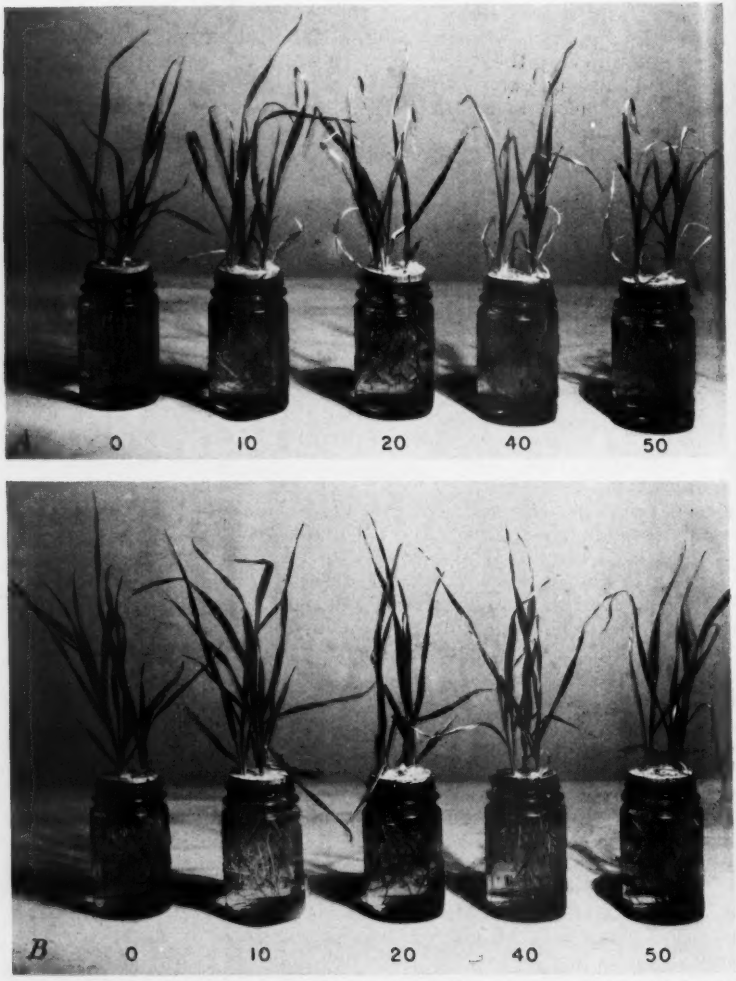

FIGURE 1.—Barley plants after 7 days in nutrient solutions containing 0, 10, 20, 40, and 50 p. p. m. of sodium chlorate: *A*, In acid solutions, pH 5.1–6.2; *B*, in alkaline solutions, pH 9.1–8.3.

those in the alkaline solution (pH 7.9–7.4) were still practically uninjured, only the tips showing slight withering. Growth of the control plants without chlorate remained good at all three reactions for the duration of the experiments and showed that the symptoms attributed to chlorate were not due to unfavorable acidity in any case.

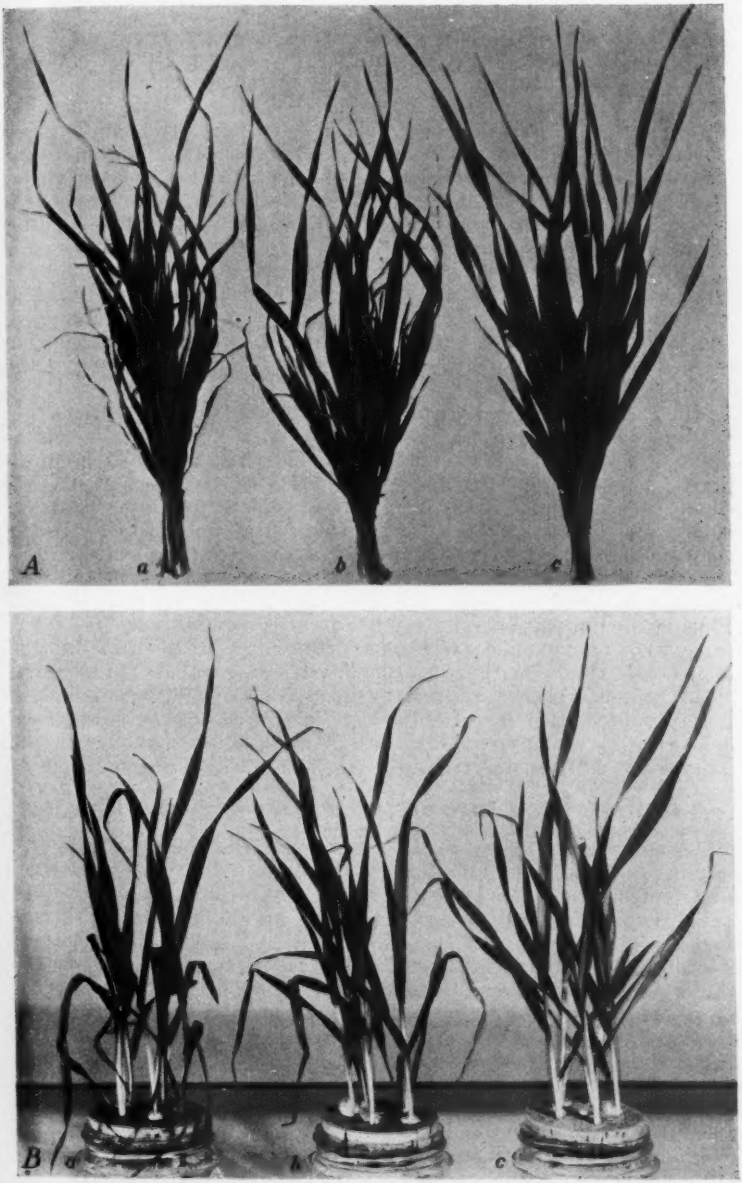

FIGURE 2.—*A*, Barley plants 11 days after the addition of 10 p. p. m. of sodium chlorate to: (*a*) Acid (pH 5.1–6.2), (*b*) neutral (pH 6.8–6.9), and (*c*) alkaline (pH 9.1–8.3) solutions. *B*, Barley plants 4 days after the addition of 40 p. p. m. of sodium chlorate to: (*a*) Acid (pH 5.0–5.4), (*b*) neutral (pH 7.0–6.6), and (*c*) alkaline (pH 7.9–7.4) solutions.

Another experiment with barley was carried out similarly with 20 p. p. m. of sodium chlorate in three solutions whose reactions ranged from pH 5.2 to 5.9, 6.4 to 7.0, and 9.2 to 8.1, respectively. After 4 days, only the plants in the acid solution showed chlorate injury—fired leaf tips and shriveled lower leaves. After 6 days, injury was severe in the acid solution, moderate in the near-neutral solution, and absent in the alkaline one. After 2 weeks, when the plants were cut and weighed, those in the alkaline solution averaged 96.5 percent as large as the corresponding controls without chlorate, as compared with 80 and 66.5 percent as large in the case of the plants in the neutral and acid solutions, respectively.

In the first soil experiment 3-week-old plants from duplicate plots of corn, soybean, millet, and sorgo all showed least chlorate injury in alkaline soil (pH 8). Other plants cut from the plots 2 weeks later gave similar results for each crop except millet, which at this cutting showed no difference between the degrees of chlorate injury in the limed and unlimed plots.

The large quantity of lime used here to produce a soil reaction of pH 8.0 was itself injurious, as shown by the smaller size of the control plants. Therefore, the experiment was repeated with half as much lime (1 pound per plot), which produced a soil reaction of pH 7.5. Again corn, soybean, sorgo, and barley were all less injured by the chlorate in the limed than in the unlimed plot at both of two cuttings, comprising alternate plants cut after 2 and 4 weeks, respectively. But in a repetition of the experiment immediately afterwards, with millet, sorgo, and sunflower, this effect of the lime was not obtained. Thus, while liming generally rendered the chlorated soils less toxic, there were unexplained exceptions. Moreover, in no case was the tendency of alkalinity to reduce the toxicity of chlorate as pronounced in soil as it had been in the water cultures. It should be emphasized that each of the soil experiments was begun with newly chlorated soil in an effort to avoid complications that would ensue if the rate of decomposition of the chlorate should be affected by the soil reaction.

PERSISTENCE OF CHLORATE TOXICITY IN RELATION TO SOIL REACTION

It was observed in one of the experiments that barley plants of a certain limed plot were taller and less chlorotic than those of an adjacent neutral plot throughout their early seedling stage, but that they lost this advantage by the time they were cut and weighed at the age of 6 weeks. Their initial superiority reflected the lesser toxicity of chlorate in an alkaline substrate. The fact that as time went on the plants became increasingly poorer than those in the neutral plot suggested an inhibiting effect of the soil's alkalinity on the rate of decomposition of the chlorate, which would accord with Yamasaki's (*20*) finding that chlorates undergo reduction more easily in acid than in neutral or alkaline substrates.

To test the relative rates of recovery of acid, neutral, and alkaline soils made toxic by sodium chlorate, some greenhouse-soil plots were acidified to pH 5.2 with sulfuric acid, others were made alkaline (pH 8.0) with hydrated lime, and still others were left at the natural reaction, pH 6.8. Sodium chlorate was then stirred into half of them, and the rest left as controls without chlorate. Black Amber sorgo was

sown the next day. Each series of plots was duplicated in two greenhouses designated A and B.

Five successive crops of sorgo were grown in these plots without further treatment. The average green weights of the plants of each of the five crops, expressed as percentages of corresponding averages for control plants in soil of the same reaction without chlorate, are given in table 1.

TABLE 1.—*Comparative persistence of chlorate toxicity*[1] *in acid, neutral, and alkaline soils*[2] *as shown by growth percentages*[3] *for successive crops of sorgo*

[Chlorate added to soil on July 24, 1939]

Sowing No.	Period of growth 1939–1940	Greenhouse	Growth percentages of chlorated plants at the following soil reactions			Average weights of control plants without chlorate at the various soil reactions		
			Acid	Neutral	Alkaline	Acid	Neutral	Alkaline
			Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
1	July 25 to Aug. 23	A	2.3	1.8	3.5	23.11	20.11	13.09
		B	1.5	1.3	3.3	19.72	19.64	13.73
2	Aug. 31 to Oct. 5	A	25.1	21.3	8.8	9.04	9.29	8.40
		B	22.4	19.5	10.7	14.80	13.32	9.54
3	Nov. 10 to Dec. 19	A	65.9	64.5	26.3	1.59	1.66	1.74
4	Dec. 20 to Jan. 22	A	102.9	106.1	64.0	.55	.50	.48
5	Jan. 22 to Mar. 7	A	135.9	106.2	51.4	1.28	1.61	1.40

[1] The toxicity of all the chlorated plots was reduced between the first and second sowings by adding with thorough mixing 80 pounds of untreated soil to that in each compartment. The control plots received similar additions.

[2] The pH values ranged over the period of the experiment from 5.2 to 5.3, 6.8 to 6.6, and 8.0 to 7.5 for the acid, neutral, and alkaline soils, respectively.

[3] Average green weights of 80 to 100 plants in terms of corresponding weights of control plants from soil of the same reaction without chlorate as 100.

The data show that chlorate toxicity was initially very severe in all the plots, reducing the plants to a small fraction of the size of the controls. Those in the alkaline soil were the least injured, as shown by somewhat higher growth percentages. In successive croppings toxicity decreased progressively in all the plots, but much less rapidly in the alkaline ones than in the others; with the result that, where the plants were least injured in the first sowing, they were definitely the most injured in all subsequent ones. When the experiment was discontinued, after five sowings, the soil of these alkaline plots was still highly toxic, while that of the neutral and acid plots had lost its toxicity completely.

The weights of the control plants showed that the lime was itself injurious for the first two sowings but not thereafter. There was no significant difference in the size of the control plants of the neutral and acid plots at any time. The decline in yield with time is without significance for the present study, being due to the decreasing size attained by plants of similar age as the season progressed from summer through the winter months.

The soil plots in greenhouse B of table 1 were not resown with sorgo after the second sowing but instead were used for a series of five successive sowings of sunflower. Each plot was divided into two sections by a board partition, and sodium nitrate, at a rate of 150 gm. to 250 pounds of soil, added to one and not the other. The comparative persistence of chlorate toxicity in the various plots is shown in table 2.

TABLE 2.—*Comparative persistence of residual chlorate toxicity in acid, neutral, and alkaline soils*[1] *as shown by growth percentages*[2] *of successive sowings of sunflower*

[Chlorate added to soil on July 24, 1939]

Soil and sowing No.	Period of growth 1939–40	Growth percentages of chlorated plants at the following soil reactions			Average weights of control plants without chlorate at the various soil reactions		
		Acid	Neutral	Alkaline	Acid	Neutral	Alkaline
		Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
Without excess nitrate:							
1	Nov. 9 to Dec. 20	51.3	28.3	14.1	45.1	44.5	28.8
2	Dec. 20 to Mar. 6	58.5	30.4	11.9	44.9	49.3	36.2
3	Mar. 7 to Apr. 15	62.2	54.4	17.6	17.0	17.7	16.0
	Mar. 7 to Apr. 29	77.1	62.5	15.5	48.4	50.4	46.2
4	Apr. 29 to May 24	106.9	76.2	27.4	18.4	20.5	22.2
	Apr. 29 to June 3	109.3	83.2	25.4	48.4	51.8	52.0
5	June 4 to June 28	123.1	108.3	49.3	12.9	12.5	13.0
	June 4 to July 8	106.9	95.8	43.8	47.5	54.9	48.0
With excess nitrate:							
1	Nov. 9 to Dec. 20	58.3	59.4	23.1	36.1	30.2	29.7
2	Dec. 20 to Mar. 6	63.6	83.9	42.0	42.7	34.6	32.8
3	Mar. 7 to Apr. 15	64.2	74.6	38.9	14.2	13.8	13.6
	Mar. 7 to Apr. 29	83.0	78.0	32.2	43.8	41.8	41.0
4	Apr. 29 to May 24	86.2	72.9	36.8	21.2	20.8	20.0
	Apr. 29 to June 3	82.2	90.1	37.0	47.7	46.7	46.7
5	June 4 to June 28	103.4	96.8	57.5	12.4	11.9	12.1
	June 4 to July 8	86.5	115.7	45.3	51.8	45.3	47.1

[1] The pH values obtained over the period of the experiment ranged from 5.3 to 5.5, 6.8 to 7.2, and 7.5 to 7.6 for the acid, neutral, and alkaline soils, respectively.

[2] Average green weights of about 20 plants in terms of corresponding weights of control plants from soil of the same reaction without chlorate as 100.

Much the smallest growth percentages, indicating greatest toxicity of the chlorate, were obtained for plants of the alkaline plots throughout. The failure of these plants to show an initial superiority is accounted for by the fact that the chlorate had already been in the soil for 3.5 months (since July 24) at the time of the first sowing, so that differential decomposition of the chlorate had already occurred—greatest in the acid, least in the alkaline soil. Toxicity disappeared completely from the acid and neutral soils by the time of the fifth sowing; but the plants in the alkaline plots were still very chlorotic at this time and were only about half the size of the control plants in the same soil without chlorate. The data show conclusively that lime greatly increased the time required for decomposition of the chlorate. The contrast in the appearance of the plants of the limed and unlimed plots by the time of the third sowing is illustrated in figure 3.

When the percentages for plants from the nitrated plots are compared with those from the plots without added nitrate it is seen that the effect of nitrate in reducing toxicity, first discovered by Crafts (*8*), was more pronounced in the neutral and alkaline soils than in the acid one. As a result, the plants of the acid plots were not superior to those of the neutral plots in the nitrated soil, thus constituting the only exception to the generalization of most rapid decomposition of the chlorate in the most acid soils.

Other experiments with sunflower and barley are summarized in table 3, both begun with newly chlorated soil.

The plants of the first sowings of both crops were somewhat less injured by chlorate in the alkaline plots than in the neutral ones. Subsequently this relation was reversed, as in the experiment of table

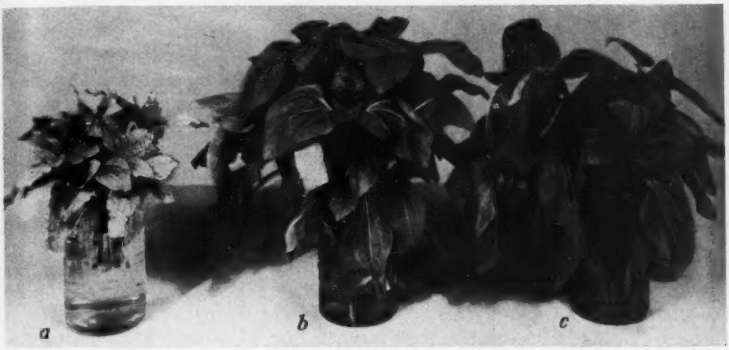

FIGURE 3.—Comparative degrees of chlorate chlorosis and stunting of 5-week-old sunflower plants grown in (*a*) limed, (*b*) neutral, and (*c*) acidified soils treated 9 months previously with equal quantities of sodium chlorate. The control plants, without chlorate, showed no effects of the soil reaction, all being normal in size and color at this time.

1 where, as here, the seed was sown within a few days after the application of chlorate. During the several-month period of the experiments, chlorate toxicity disappeared completely from the acid soils, and was much reduced in the neutral soil, but it changed much more slowly in the alkaline one. Concomitantly, leaf chlorosis, equally severe on all the plants at first, disappeared completely from the acid plots but persisted with almost unabated severity in the alkaline plots to the end. Plants of the neutral plots were clearly intermediate after the first sowings. The color of all the control plants was normal.

TABLE 3.—*Comparative persistence of chlorate toxicity in acid, neutral, and alkaline soils* [1] *as shown by growth percentages* [2] *of successive croppings of barley*

[Chlorate added to soil on Jan. 2 and Mar. 11, 1940, for experiments with barley and sunflower, respectively]

Crop and sowing No.	Period of growth	Growth percentages of chlorated plants at the following soil reactions			Average weights of control plants without chlorate at the various soil reactions		
		Acid	Neutral	Alkaline	Acid	Neutral	Alkaline
		Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
Barley:							
1	Jan. 3 to Feb. 14	12.6	8.9	13.4	4.07	3.96	3.89
2	Feb. 16 to Apr. 1	15.3	7.9	7.2	11.27	11.54	10.50
3	Apr. 1 to Apr. 30	40.8	21.9	17.9	2.75	2.86	2.94
4	Apr. 30 to May 23	81.9	53.8	33.4	2.30	2.08	2.43
5	May 23 to June 21	140.0	76.9	52.1	2.25	2.98	3.14
Sunflower:							
1	Mar. 12 to Apr. 5	50.3	30.9	41.6	8.49	7.07	4.98
2	Apr. 8 to May 3	73.0	50.4	41.0	11.11	10.32	6.50
3	May 3 to May 27	99.7	77.7	47.8	12.60	11.96	8.63

[1] The pH values obtained over the period of the experiments ranged from 5.3 to 5.5, 6.8 to 7.0, and 8.1 to 7.6 for the acid, neutral, and alkaline soils, respectively.

[2] Average green weights of 75 to 85 plants of sunflower and at least 100 of barley in terms of corresponding weights of control plants from soil of the same reaction without chlorate as 100.

There is a further relation that should be pointed out in connection with the first sowings of tables 1 and 3 (comprising all the experiments begun with newly chlorated soil), namely, that the plants of the acid plots were not the most injured, as would be expected, but were less injured than those of the neutral plots in every case. This disagreement with the direct relation that obtained in the water cultures is probably attributable to the effect of acidity on decomposition of the chlorate by reducing agents of the soil, superimposed on the direct effect of acidity on chlorate injury to the plant. This point will be discussed later.

The occurrence of growth percentages well above 100 for final sowings, such as those of the acid plots of tables 1 and 3, show that after chlorate toxicity had disappeared the plots produced larger plants than did the adjacent control plots of the same reaction without chlorate. Several investigators have reported such instances of apparent stimulation of crops by chlorate. Strobel and Scharrer (*19*) and Bissey and Butler (*5*) have observed it with wheat, and Bowser and Newton (*6*) with flax, although the latter attributed it to elimination of weeds by the chlorate. Stimulation of sorghum was observed by Timmons (unpublished report, 1939) who stated (p. 20) that "there was a definite tendency for the yields of most of these crops [sorghums] to be higher on the treated areas than on check plots, probably due to the moisture conserved by the absence of growth on the treated areas the previous year or two." Conservation of nutrients would also be expected where chlorate has previously reduced plant growth. Bissey and Butler (*5*), however, found that the apparent stimulation observed by them did not result from reduction of fertility in the check plots, for these remained equally productive over the period of experimentation. In the present investigation relatively greater exhaustion of the soil in the control plots by the end of each experiment can be assumed but it cannot be proved from the data available. It seems to the writer that another explanation may be found in a possible destructive effect of chlorate on the deleterious soil organisms to which crops like sorghum are particularly susceptible.

CELL-SAP ACIDITY AND SUSCEPTIBILITY TO CHLORATE INJURY

The effect of the reaction of the substrate on chlorate toxicity suggested the possibility of a relation between plant acidity and degree of susceptibility to chlorate injury which varies widely with different crops (*12*). Although reports found in the literature (*11*) showed no acidity differences that could be correlated with differences in susceptibility to chlorate, it seemed desirable to compare the acidity of some of the crops when grown side by side in the same greenhouse.

Some 200 pH determinations [3] on the expressed juice of leaves of 15 different crop plants were made with a Leeds and Northrup glass-electrode apparatus checked frequently with standard buffer solutions. A comparison of these determinations showed that differences in susceptibility to chlorate injury cannot be explained on the basis of differences in plant acidity. Thus, pH values for four varieties of

[3] These determinations were made by A. L. Pitman, of the Industrial Farm Products Research Division Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

barley, one of the most susceptible crops, ranged from 5.91 to 6.27, as compared with a range of 5.88 to 6.05 for four varieties of oats, a highly resistant crop; and pH values for spinach, which generally proved most susceptible of all to chlorate, ranged from 5.84 to 6.24, as compared with a range of 5.80 to 5.89 for the highly resistant flax. Obviously some factor other than sap acidity is responsible for the differences in the severity of chlorate effects on these plants.

DISCUSSION

The toxicity of a given concentration of chlorate tends to vary directly with the hydrogen-ion concentration of the substrate. However, from the rather small effects and the occasional exceptions obtained in the soil experiments of the present investigations it seems likely that the relation is of minor importance for the problem of chlorate treatment of soils for weed control. More important seems the fact that chlorate toxicity disappears so much more slowly from limed soil than from near-neutral and acidified soils. This finding, if confirmed under field conditions, would indicate a distinct disadvantage in the use of chlorate on alkaline soil, for to the tendency of the chlorate application to be least toxic, hence least effective in killing the weeds, is added the even more serious objection of subsequent slower recovery of such soils for succeeding crops. Presumably the explanation of the greater persistence of chlorate toxicity in limed soil is to be found in the relatively slow decomposition of the chlorate at alkaline reactions.

Whenever an experiment was begun with soil to which chlorate had been added several months previously (in which presumably the differential decomposition due to soil reaction would already have occurred) the data always failed to show the initial superiority of chlorated plants in limed soil that would be expected from the fact of their superiority in alkaline nutrient solutions.

The plants of acid soil plots were less injured by chlorate than were those in neutral plots. This reversal of the direct correlation of toxicity with degree of acidity, found to obtain in water-cultures of both the present and previous investigations (*10, 17, 20*), may be explainable on the basis of more rapid decomposition of chlorate in the acid soil—a reaction not occurring in culture solutions of similar acidity because of lack of reducing agents. As stated by Offord (*16*), "The high oxidizing potential of sodium chlorate and the readiness with which this compound gives up its oxygen when intimately associated with organic matter is a well-known chemical fact."

In explanation, then, of the relation between soil reaction and persistence of chlorate toxicity, it can be assumed that the rate of decomposition of chlorate to the relatively harmless chloride, a process known to occur in the soil and to be affected by various conditions (*2, 3, 6, 9, 14*), is favored by acidity and hindered by alkalinity, as might be expected from its chemical properties (*15, p. 313*). In accord with this explanation is Yamasaki's finding (*20*) that glucose and aldehydes can reduce chlorates when the solutions are acid but not when they are neutral or alkaline. Since one of the advantages in the use of chlorates for weed control is their relatively rapid disappearance from treated soils, their persistence under alkaline conditions would indicate the need of caution in applying them to limed soils,

and possibly to the alkaline soils of the western part of the United States. Certainly, to apply lime in order to hasten recovery of the soil after chlorate has been used to kill weeds, an idea that had been suggested by the tendency of chlorate to be least toxic in alkaline solution cultures, can now be expected to greatly prolong the toxic condition. The best recovery of an alkaline soil by the time all toxicity had disappeared from adjacent acid plots was to 64 percent of normal (table 1). Usually recovery was to about 50 percent of normal.

Incidentally, it is of interest to note in this connection Yamasaki's idea (*20*) that the active agent in chlorate toxicity is the hypochlorite ion produced on reduction of the chlorate to hypochlorite within the plant. He reports that susceptibility to chlorate is actually greatest in those plants that are highest in such reducing substances as glucose and aldehydes. If the reduction takes place only in acid solutions, it might be assumed that the more acid the plant the greater its susceptibility to chlorate. Yamasaki actually found that growing plants in acid solutions increased their susceptibility to chlorate and assumed the effect to be due to an increase in their sap acidity. However, he found no correlation between the acidity measurements for seedlings grown under ordinary conditions and their relative susceptibility to chlorate injury. Nor could any such correlation be found in the present investigation.

Arenz' finding (*1*) that barley is high in reducing capacity, as compared with lupine, should be noted in this connection, for barley is one of the most susceptible crops to chlorate. Also of interest, in view of the extreme susceptibility of spinach, is Stoklasa's finding (*18*) that the Chenopodiaceae in general are high in oxalic acid. However, the hydrogen-ion concentrations obtained by Stoklasa, the present writer, and others (*11*) for the expressed juice of spinach are no higher than those of more resistant crops.

The assumption is made throughout this paper that the effect of lime on the persistence of toxicity of chlorate-treated soils is an effect of alkalinity. However, a puzzling aspect presents itself in the fact that so large an effect persists in the limed soil after its alkalinity has decreased through successive sowings to values as low as pH 7.5, as in the experiment summarized in table 1. Furthermore, it is surprising that there should be such an extreme contrast in the toxicity of chlorate in soils whose reaction differed no more than did that of the "neutral" and alkaline soils of table 2, the former ranging, between the beginning and end of the experiments, from pH 6.8 to 7.2 and the latter from pH 7.5 to 7.6. There is a suggestion here that the action of lime in delaying the disappearance of chlorate toxicity from soils may be some effect other than that of alkalinity. On the other hand there is the fact that acidifying the soil could hasten the disappearance of toxicity as outstandingly as liming delayed it, which would point to degree of acidity as the determining factor. Certainly such pronounced and regular trends of declining growth percentages with decreasing degrees of soil acidity as those shown by table 3 would seem to justify such interpretation.

SUMMARY

The toxicity of sodium chlorate to soybean, sunflower, and barley in water cultures generally varied directly with the acidity of the nutrient solution, being least at alkaline reactions and greatest at acid

ones. Decreasing the soil acidity of greenhouse plots to about pH 8.0 with lime sometimes, but not always, decreased the toxicity of newly added chlorate.

Chlorate toxicity disappeared much more slowly from limed than from unlimed soil. In general, it disappeared most rapidly from acidified soil, but the difference between the rates of recovery of the acid and neutral plots was usually less marked and less consistent than the difference between these and the alkaline plots.

In consequence of the dual effect of acidity—on the toxicity of chlorate and on its rate of decomposition—alkaline plots that were the least toxic immediately after chlorate was added were the most toxic to subsequent crops. This reversal of the initial relation between limed and unlimed plots is believed to be due to an effect of soil reaction on the rate of decomposition of the chlorate, a process hindered by alkalinity and favored by acidity.

Since a given concentration of chlorate tends to be least toxic in alkaline soil, hence a less effective poison for weeds, and at the same time is a more lasting poison for succeeding crops, a question is raised as to the advisability of applying it to alkaline soils.

There was no correlation between the pH value of the expressed juice of various crop plants and their relative susceptibility to chlorate injury.

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